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Art. XII.—*Studies of the Varieties of Subterranean Clover.*

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Introduction.

In Australia, the occurrence of varieties within the species of *Trifolium subterraneum* was first noted by Adams of Muresk College, Western Australia in 1924, and several varieties were described by him from 1924 to 1934 (1–7).

In Victoria, on the initiative of Mr. J. E. Harrison of the Department of Agriculture, a few samples obtained from each of the southern states of Australia were grown for observation in 1928. This preliminary work showed that a wide diversity of types existed and indicated the necessity for making a more extensive collection. This was arranged by Mr. Harrison, and largely with the assistance of district dairy supervisors of the Victorian Department of Agriculture, a State-wide collection was assembled in the summer of 1928/29. Officers were requested to collect samples from fields in which the clover had appeared voluntarily, rather than from fields which had been sown with commercial seed. This procedure, which was based on the knowledge that most commercial seed was of the one type, was adopted in order to overcome the possibility of assembling too cumbersome a collection, without reducing the collection of lots likely to show differences. With the addition of further samples from Western Australia, South Australia, Tasmania and the United States of America, 143 lots were grown in 1929. To these were added collections from Europe and New Zealand in 1930 and 1931, while new samples from Australian sources have been collected since and grown each year.

In 1929, owing to the absence of Mr. Harrison, the first large collection was examined and classified by Mr. F. R. Drake of the Victorian Department of Agriculture, who continued his observations on selected units of this and other collections in the following years.

The most noteworthy feature of the first and subsequent collections was the remarkable uniformity shown by the plants grown from any one sample. In only eight lots of the first large collection was there any marked lack of uniformity and even these consisted of a mixture of only two or three distinct types. Approximately half of the same collection proved to be of the standard commercial type, generally described as a mid-season type or "Mt. Barker." The remaining lots, which included some very extreme types, differed from this standard type in one or more characters and provided sufficient material for a classification into early, mid-season, and late flowering strains (Plate XVIII). Differentiations were made within each of these groups according to minor variations in dates of flowering, and to such characters as habit of growth, location of first flower on the runners and colour or markings of stem, leaf, flower and seed. On this basis 39 strains were separated and noted in 1929, and though fresh collections have added to this number from time to time, the 1929 collection proved to be the largest and most fruitful source of variable material. The strains have been grown as separate plants in adjacent plots from year to year, and have exhibited an extreme stability of type. The practical aspect of the wide variations between strains has commanded most attention, and has led to the commercial development of a few very promising pasture strains.

In 1936, Miss Y. Aitken began a study of the subterranean clover collections, in order to formulate a scientific record of the strains which had been isolated, and to study their response to such environmental factors as length of day, temperature and saturation deficit.

The Type Variety of the Species in Australia, and its Variation, Phenotypical and Genotypical.

In Australia the most widely spread variety is that known as Mt. Barker. A description of an individual plant grown under standard conditions (fig. 1), and of its capacity for phenotypical and genotypical variations, gives a basis on which the known polymorphy of the species can be recorded and analysed; such a description is as follows.

A prostrate, villous, annual plant with a short main axis and strong lateral growth.

Seedling.—Epigeal, cotyledons glabrous, 5 mm. long, their stalks twice as long as their oval blades.

FIG. 1.—Stages in the development of a plant of *T. subterraneum* —variety "Mt. Barker".

Growth and flowering.—Each plant develops about five basal leaves, and then grows into its winter phase the rosette state, then from the basal nodes, about five prostrate runners are produced, each of these runners has about three nodes. In late winter with the onset of flower development (fig. 2*a*), the youngest internodes of the runners elongate slightly, while secondary laterals begin to develop from the basal nodes of the runners. By early September, the internode proximal to the first flowerhead elongates conspicuously, and by mid-September, the first flowerheads open. These develop at about the tenth node of each runner. Later, axillary flowerheads form at the more distal nodes and also occur on the secondary laterals. Towards the end of the flowering period, after about nine flowerheads have formed along the runner, the later-formed internodes and peduncles are dwarfed, and few of the corresponding flowerheads form viable seed. After the formation of about sixteen runners, flowering at the top of the main axis prevents further runner initiation. According to Wexelson (25) the chromosome number in the vegetative stage is 16.

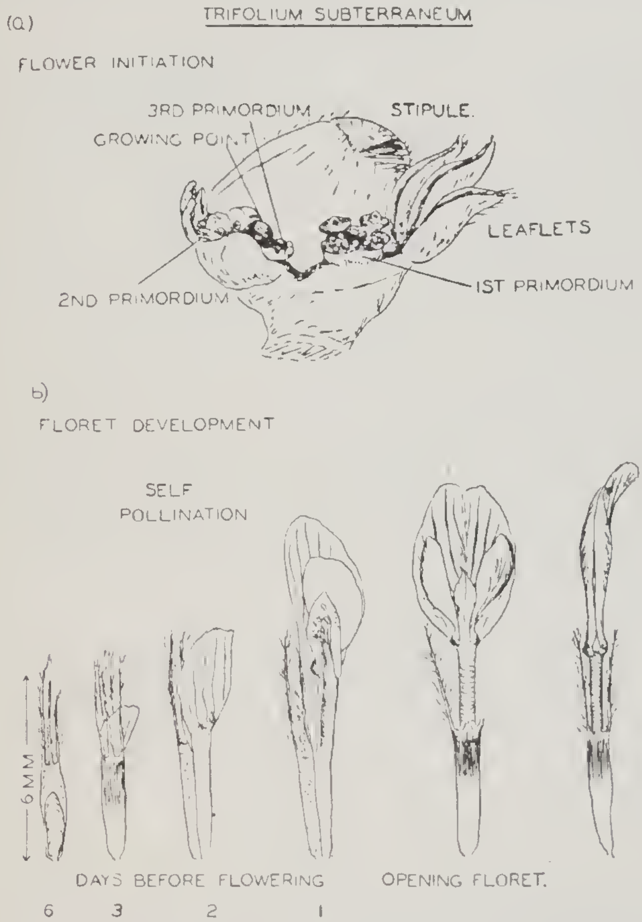

FIG. 2.—Growth of the flower (*a*) the tip of a runner, dissected to show the flower primordia, shortly after initiation; (*b*) the appearance of a floret, several days before and after self-fertilization.

Leaves.—First leaf simple, villous, about 9 mm. long and 12 mm. broad, its base almost straight; later ones ternate, leaflets obcordate, villous on both surfaces, green with local brown flecks due to anthocyanin in the upper epidermis. Centrally across each leaflet is a pale green crescentic area, which becomes less conspicuous in the late flowering stage. The petiole is villous, and four or more times longer than the leaf. The stipules are more or less villous, ovate-acute, attached in their lower halves to the petiole, typically red-striped over the veins and often red between the veins also.

Stems.—The stems of the runners are villous, and green turning to brownish in spring, with exposure to sunlight as the internodes lengthen.

Inflorescence.—Flowerheads axillary, each with three to five perfect florets, peduncle 1.5 cm. long. Floret about 12 mm. long; calyx tubular, yellowish below, red on half to two-thirds of the upper part of the tube with five green, narrow, free setaceous lobes a little longer than the tube; corolla white, with faint pink veins on standard and alae; standard three times as long as the calyx tube. Self-fertilized, pollination occurring when the tip of the folded corolla is at the level of the tips of the calyx lobes (fig. 2*b*). The standard and alae expand fully two days later, and then the corolla withers, the pedicels become reflexed while the distal region of the peduncle becomes positively geotropic and bends down to the ground and lengthens up to 4–5 cm., thus forcing the developing fruits into the ground if soft. The peduncle tip forms a succession of four- or five-rayed to simple pronglike growths which are, morphologically, sterile and partly-developed flowers, these turn upward round the developing fruits and thus form the burr characteristic of the species. Each burr usually contains three to four seeds. Ovary with two anatropous ovules, only one developing to form a one-seeded fruit with a brown, membranous wall.

Mature seed.—Purplish-black, dull-surfaced, oval except for the radicle notch, $1\frac{1}{2}$–$2\frac{1}{2}$ mm. long.

The characters entirely specific in the genus to *T. subterraneum* are (1) an inflorescence of very few perfect florets (2–5); after fertilization (2) the development of many spur-like sterile florets and (3) the occurrence of elongation and positive geotropism in the peduncle.

Polymorphy within the Variety.

Phenotypical variation in such environmental factors as light intensity, temperature, competition and disease incidence may alter the appearance of the plant in several ways.

(*a*) Internodal length, the number of laterals along a runner, and the number of runners may be reduced by competition (see fig. 3*a*; Plate XVII., fig. 2) by insufficient water or nutrients or by disease. A small, sparse-leafed plant results. Extreme conditions reduce the rate of node formation.

Other things being equal, the date of germination influences the position at which the first flower is produced on the basal runners, and also the development of laterals (fig. 3*b*).

(*b*) The length of petiole and the size of leaves are also reduced by insufficient water-supply and by disease.

(*c*) The brown flecks and shading on the leaves and stem surfaces are prevented or reduced by shading from direct sunlight, or by mosaic infection. Attacks by the red spider cause a general red appearance of the leaves; while phosphate deficiency results in a purplish brown colour.

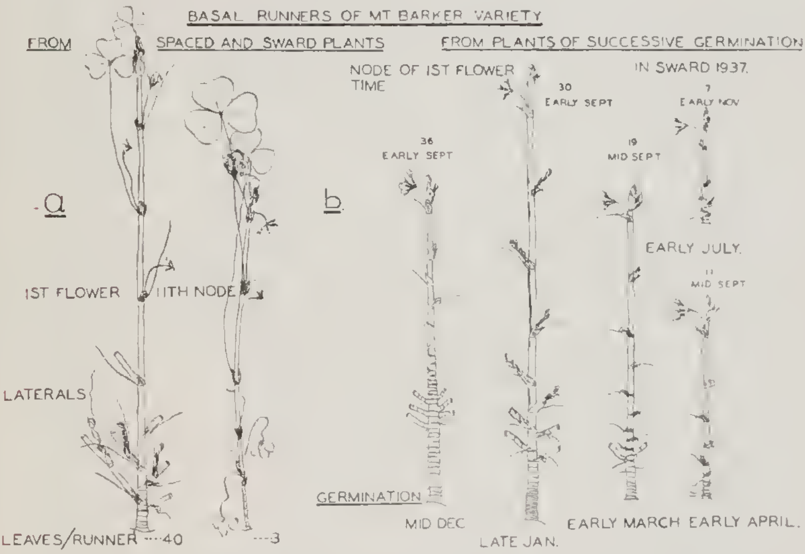

Fig. 3.—Basal runners of "Mt. Barker" variety, showing (A) the effect of sward, and spaced conditions, on lateral and leaf production; (B) the effect of date of germination in a sward, on lateral production, and on the node at which the first flower was produced.

Other abnormalities such as a marked dwarfing of the plant and a conspicuous reddening of the leaves have occasionally been noted, but not yet investigated.

Genotypical variations within the type variety are few owing to the close selfing of each plant. Probably the strains "White-Seed" and "Amber-Seed," may be regarded as variations due to a bud-mutation, preventing the development of the usual purple-black colour of the testa and of other anthocyanin markings in leaves, stem and flower. Such a bud-mutation certainly occurred on a plant of the variety Dwalganup when a burr with white seeds was found on a runner bearing burrs with normally coloured seeds. The following year the white seeds produced plants of the Dwalganup type but without anthocyanin in any part. "White-Seed" and "Amber-Seed" differ similarly from "Mt. Barker" and so also does a white seeded plant which occurred in the 1940 plot of the variety "Bass." Variations from the normal recorded in the following section, may be due to similar mutations though no direct evidence has yet been found.

Polymorphy within the Species.

This survey of the hereditary variation within *T. subterraneum* shows a close similarity with that found in other closely-studied species of the Leguminosae. In Table 1, the variation of homologous characters of *T. subterraneum* is compared with those of *Pisum sativum* and *Vicia sativa,* after the plan of V. Muratova (17) and with the use of his data on *Vicia sativa* and with local data on *P. sativum.* Agriculturally it is particularly significant that forms occur with wide variations in respect to such important vegetative characters as length of vegetative period, lateral formation and size of leaf.

It should be noted also, that the record of variations within the other two species are the result of the study of an exhaustive world collection, whereas that for *T. subterraneum* is based mainly on Australasian types. Hence the similarity in homologous variation may be expected to become even closer when more European forms have been collected and studied.

At Burnley Gardens, Melbourne, observations of the various samples grown under the same conditions year after year, have shown that more than 50 varieties exist. Each variety differs from the rest by at least one distinct character. Table 2 lists the main characters of each variety.

Discussion of Major and Minor Characters.

Major Characters.

Major characters are those responsible for the detailed growth form and biological efficiency of the plant. These are:—

1. Number of runners per plant.
2. Lateral development (number of laterals per runner and degree of branching).
3. Internode length.
4. Leaf size and petiole length.
5. Number of seeds produced.

The number of runners per plant, the number of laterals and their degree of branching (figs. 4*a*, *b*) are strongly influenced by the inherited character of time of flowering, which may be considered to be an inter-related major character (Plate XVIII). The numerical production of stem growths (runners and laterals), which depends on these characters, largely determines the plant's capacity for the production of leaves and seed, as in the axil of each leaf a vegetative shoot or a flower may be produced. The four or five first produced, or "basal" runners

TABLE 1.—LIST OF THE HERITABLE CHARACTERS KNOWN TO OCCUR IN *T. subterraneum*, *Vicia sativa* and *Pisum sativum*.

—		T. sub.	Vicia sativa.	Pisum sativum.
FRUIT—				
Size	small	..	..	..
	medium	+	+	..
	large	+	+	..
Number of Seeds	1	+	..	..
	2	+	..	..
	3–6	..	+	+
	7–12	..	+	+
SEED—				
Colour	white	+	+	..
	yellow	..	+	+ (12)
	green	..	+	+
	grey	..	+	..
	dull brown	+	+	..
	red brown	..	+	+
	brown	..	+	..
	black	+	+	..
Size	small	..	..	+
	medium	+	+	..
	large	+	+	..
FLOWER—				
Corolla Colour	white	+	+	
	yellow	..	+	..
	pink	+	..	..
	purple	..	+	..
	violet	+ (19)	..	..
Calyx Colour	green	+	+	+
	with anthocyanin	+	+	+
INFLORESCENCE—				
Number of Flowers	1	..	+	+
	2–3	+	+	+
	4–7	+	..	..
VEG. CHARACTERS—				
Formation of Laterals	feeble	+	+	..
	strong	+	+	+
,, ,,	in axils of basal leaves	..	+	..
	scattered	+	..	..
STEM—				
Stem	long	+	+	+
	medium	+	+	..
	short	+	+	+
,,	pubescent	+	+	..
	glabrous	+	+	..
LEAFLET—				
Diameter	10–15 mm.	+	..	..
	15–25 mm.	+	+	+
	25–30 mm.	+	+	+
	30–40 mm.	..	+	..
Colour	anthocyanin	+	..	+
	none	+	+	+
Petiole	glabrous	+	..	+
	pubescent	+	+	..
Petiole	anthocyanin	+	..	..
	none	..	+	+
Stipule	anthocyanin	+	+	+
	none	+	..	+
,,	glabrous	+	+	+
	pubescent	+	+	..
BIOLOGICAL CHARACTERS—				
Vegetative period	early	+	+	+
	late	+	+	+

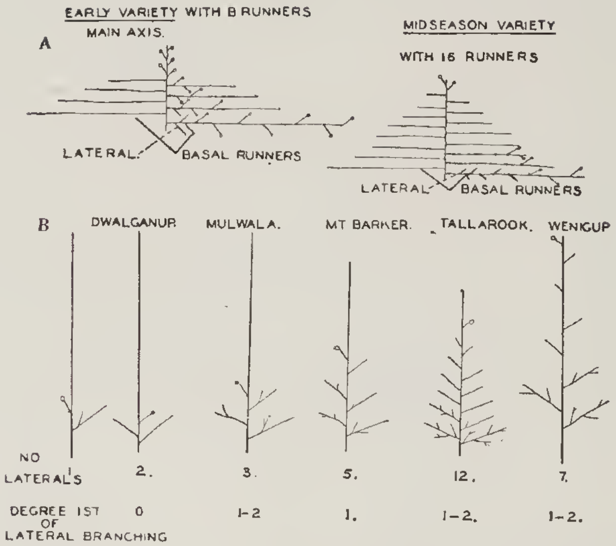

Fig. 4.—Diagram to show (*a*) the relation between the number of runners produced per plant, and the time of flowering (maturity) of a variety (main axis much lengthened); (*b*) types of runner structure, resulting from variations in number of laterals and degree of branching.

FIG 5

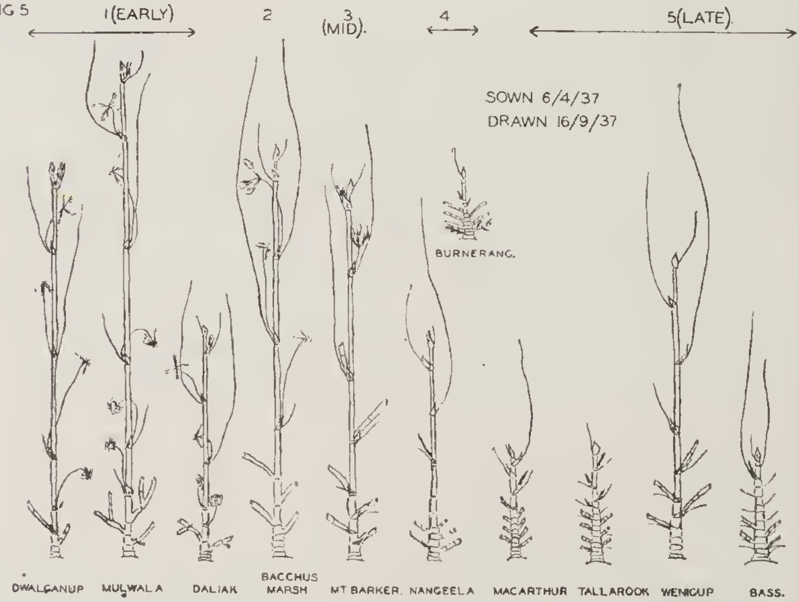

Fig. 5.—Typical basal runners of varieties sown in early April, and drawn in mid-September (laterals are not drawn in detail).

of a plant have been taken as typical, and observations and measurements confined to them. The combined effect of the major characters as above (except number of runners per plant) results in what may be termed a "basal runner organization" typical of each variety (fig. 5; Plate XVII., fig. 2).

1. Characters of the Basal Runner.

Time of flowering is a variable character influenced by time of germination, but if all the varieties are started together the relative time of flowering constitutes a reliable expression of the length of the vegetative period of each variety. As the rate of node formation per runner is approximately the same in all varieties during active growth, except towards the end of flowering, it follows that time of flowering determines the node on which the first flower is formed along the runner. This in turn determines the capacity of the variety for lateral formation, as lateral shoots only develop below the first flower of a runner. Time of flowering is also related to the time of commencement of internode elongation, and the length of internodes during flowering. At Burnley, the date of commencement of flowering in the varieties ranges from early August to late October, but flowering dates coincide for many varieties, and for the sake of convenience the varieties may be separated into early, early mid-season, mid-season, late mid-season and late groups.

In Table 2, the varieties are listed from left to right in order of time of flowering. Reference to lines 26 and 15 shows that the number of the node on basal runners at which the first flower is formed, and the number of laterals per runner, increase with later flowering; while the internode length (line 14) following the fourth flower along the runner, tends to decrease. This character-complex is bound up with the photoperiodic response of the plant, which will be discussed later.

2. Development of Laterals.

This varies both in respect to their number and their degree of branching. Increasing lateness of flowering (as in (1) above) causes a range from 4th to 20th in the nodal number of the first flower, and from 1 to 16 in the number of laterals per runner. The first two or three, and sometimes more nodes, do not produce laterals, with the result that within the same time of flowering there is variation in number of laterals produced. The degree of branching of the laterals tends to increase with lateness of flowering (fig. 4*b*) and here again there is variation within the same maturity groups. Thus choice is possible of the most productive variety for a given length of growing season.

The variety Mulwala develops two or three double branching laterals per runner, but Dwalganup forms only one or two simple laterals. Tallarook with about ten laterals branching to the second or third degree differs markedly from Wenigup, with only about five unbranched laterals. Burnerang has an extreme capacity for lateral formation and branching, even compared with the latest varieties.

3. The Commencement of Internode Elongation.

This occurs in the terminal internode at the same time as flower initiation at the growing point, but it is usually slight till the internode before the appearance of the first flowerhead, when the internode is from three to six times longer than the basal one. In consequence, though flower initiation in the varieties ranges from late May to early September, the conspicuous lengthening of runners does not begin till one or two weeks before flowering, except in two late varieties, " Rostock " and " Wenigup." In these, conspicuous elongation occurs more than a month before flowering. The length of the internode above the fourth inflorescence along the main stem of a basal runner is taken as a measure in line 14, of Table 2, because this was found to be the most satisfactory index of this character. The length of internodes at flowering, varies from about 5 cm. in the early varieties, to less than 2 cm. in the late ones. This results in the " stemmy " appearance of most of the early varieties compared with the increasingly compact later ones. In addition, the increase of laterals and hence leafiness, emphasizes the contrast. Three exceptions are " Daliak "—a compact early variety with short internodes, and short-stalked, small leaves, and Wenigup (Plate XVIII., fig. 12) and Rostock—" stemmy " late varieties with extremely long internodes and long-stalked, large leaves. The variety Burnerang is unique in its " bunched " appearance, which is due to a combination of extremely short internodes, both before and after elongation, and very profuse lateral-formation and branching. This results in the compressed laterals bending upwards and thus raising of the leaf level in the middle of the plant (Plate XVIII., fig. 15).

4. Leaf Size and Petiole Length.

The length of petiole and size of leaf tend to decrease with later-flowering varieties, but Wenigup and Rostock are exceptions in the late group, and Daliak in the early group.

5. Number of Seeds Produced.

The number of seeds formed per flower cluster, varies normally from three to four, but in " Reigert's White-seeded," normally only three flower primordia are initiated and develop into three-flowered clusters which ripen three large seeds. In Wenigup the flower primordia vary from two to four and two to three seeds are usually formed. In Burnerang, a high percentage of " twin " seeds are formed from the equal development of the two ovules in one or two ovaries of each cluster. resulting in four or five seeds per burr.

Minor Characters.

In addition to those variations in the above characters, which are regarded as having an important bearing on their agricultural significance, the varieties usually differ in minor characters such as anthocyanin development, leaf crescent, density and pubescence, etc. The varieties are still named after the district from which each was first collected, even though other varieties may occur in the same district. Exceptions have been made for several varieties which differ from one or other of the district varieties only in a minor character, which can be indicated in the name, e.g. Pink flowered, Red leaf (Plate XIX., fig. 1), White seeded and Amber seeded.

The existence of a record of character combinations should obviate any confusion associated with locally named varieties. It is interesting to note the amount of variation already found in some characters. Anthocyanin development in the leaf lamina may vary widely in amount, and its location may be any one of the types shown in fig. *6b*. In the stipule (fig. *6c*), it may be absent or may cause red striping over veins or diffuse red areas. In the stem it causes a brownish green to dark brown appearance according to the number of epidermal cells affected; in the corolla, pink-veined white florets to dark pink ones; in the calyx (fig. *6d*) a pink or brown tipped, or red-banded to red calyx tube; in the seed a black testa; in the germinating seedling (fig. *6e*), a brown band on the hypocotyl. Its occurrence on the leaf may not be linked with its presence elsewhere, e.g. in the variety "Reigert's White-seeded" where it occurs conspicuously in all parts of the plant, except the seed testa. Conversely, absence of anthocyanin from all vegetative and floral parts is linked in several cases with a colourless testa, "White-seeded Dwalganup" and white and amber-seeded forms of "Mt. Barker."

The central pale green area, known as the "leaf crescent" in Mt. Barker, is absent from some varieties and in others varies from a central dot to a crescent stretching to the leaf edges (fig. *6a*). It may be combined with the character causing white arms in the crescentic area (fig. 6, C4) in which case, the leaf crescent is very conspicuous (Plate XIX., fig. 2). The white area on the leaf surface is caused by the presence of air below the epidermis, which is separated locally from the palisade layer.

The combination of a leaf crescent with anthocyanin modifications, makes distinctive leaf markings for certain of the varieties, e.g., in Dwalganup, Second Northam, Reigert's White-seeded, Bacchus Marsh, Mt. Barker, Nangeela, Macarthur, Tallarook, etc.

The degree of hairyness is of special interest as so many of the early descriptions of the species describe it as "very hairy" (Table 3). Certain of the early flowering varieties are pubescent over all vegetative parts, and the length, density, and laxness of

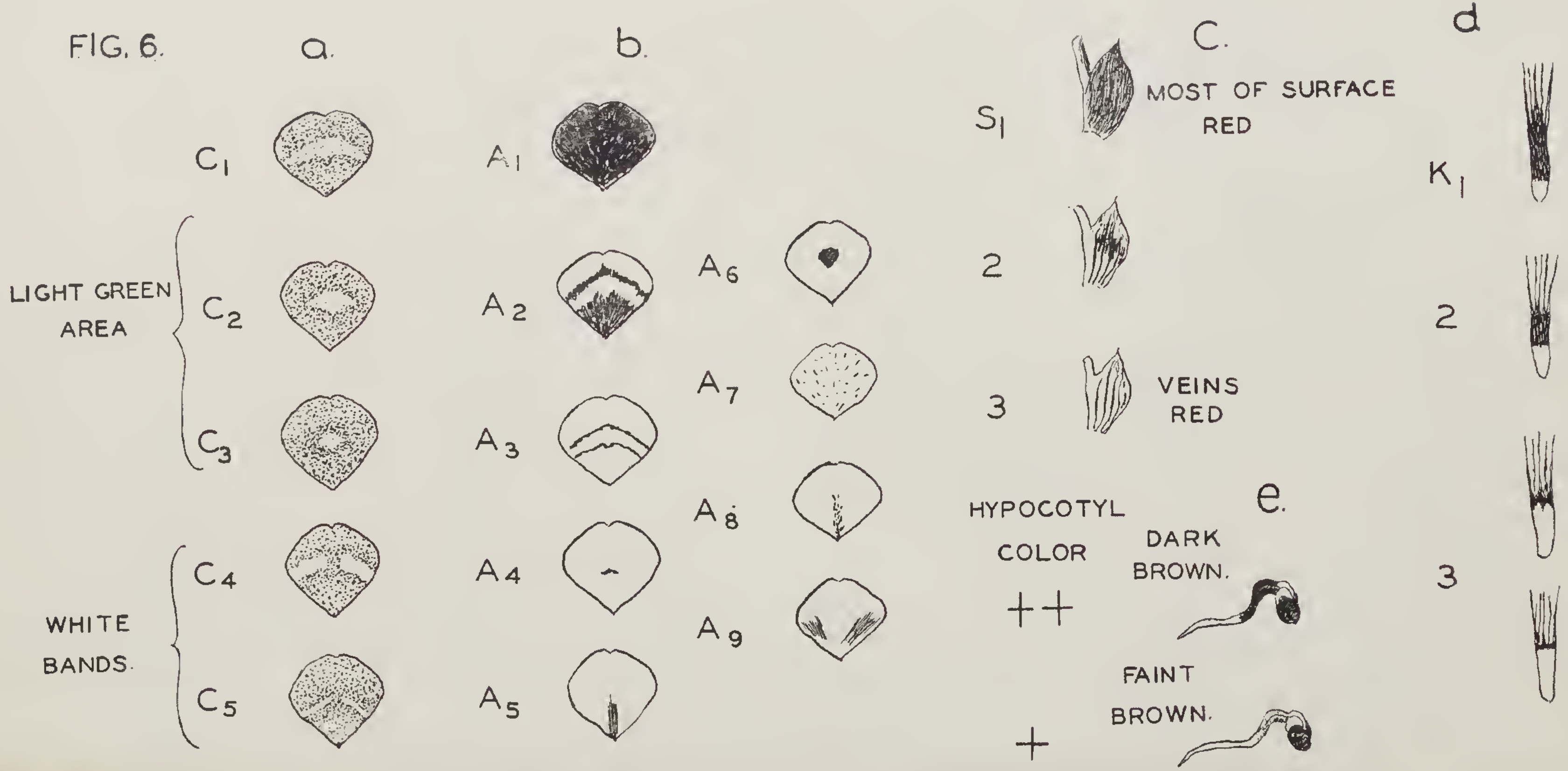

Fig. 6.—Inherited variations in the anthocyanin developed on leaf, stipule, calyx, and hypocotyl.

Table 2

E. group (flowering mid-August to late August)

Character	DWALGANUP	WHITE SEEDED DWALP	MURESK	2nd NORTHAM	MULWALA	DALIAK	NORTHAM	REIGERT'S WHITE	PINK FLOWERED	YABBA N.	SPRINGHURST	SEATON PARK
1 LEAF COLOUR of LAMINA — Grey Green	+	+	+	+	+		+		+	+	+	
2 Mid-Dark Green						+		+				+
3 PATTERN of LAMINA — Pale Central Mark	C3 5	C3 5	C3 5	C1 2	C3 5	-	C1	C4	-	C1	C3 5	C3 5
4 Anthocyanin Upper Sur.	A2 7	-	A5	A3	A5	A7	A2	A2	A7	A2 7	A2 7	A4
5 Anthocyanin Lower Sur.	A6 7	-	-	A7	-	A7	A6	A9	A7	A7	A6 7	-
6 HAIRINESS — Upper Surface	++	++	-	++	++	+	+	F	+	++	+	F
7 Lower Surface	++	++	++	++	++	+	+	+	+	++	+	+
8 *SIZE — Diameter of Leaflet	S	M	S	M	M	S	S-M	L	M	S-M	M	M
9 PETIOLE — *Length	M	M	M	M	M	S	M	M	M	M	M	M
10 Colour	B	G	B	B	B	Gb	Bg	B	B	Gb	B	B
11 Hairiness	+	+	F	++	+	F	+	-	+	-	+	+
12 STIPULE — Colour	S3	-	S2	S3	S3	S1	S3	S1 2	-	S3	S3	S3
13 Pubescence	F	+	F	+	F	+	+	-	F	-	+	+
14 *INTERNODE — Length above 4th Flower	L	L	L	L	L	M	L	L	L	M	L	M
15 *LATERALS — Number per Runner	1-2	1-2	1	2	3	2	2	2	1-2	4	2	2
16 Degree of Branching	0-1	1-2	1	1-2	1	1	1	2	0-1	1	1	1
17 *RUNNER — Number per plant	S	S	S	S	S-M	S	S	S	S	S	S-M	S
18 STEM — Colour	B	B	B	B	Bg	B	Bg	B	Bg	Bg	B	B
19 Pubescence	++	++	++	++	++	F	+	-	+	-	+	+
20 Thickness			*	*								
21 FLOWER COLOUR — Corolla Bud	P	[W]	P	W	P	W	P	W	R	P	P	W
22 Open Corolla	P	[W]	P	W	P	W	P-W	W	R	W	W	W
23 Calyx Tube	K4	-	K2	K2	K4	K1	K3-4	K4	K4	-	K4	-
24 Calyx Lobes	B	G	B	G	B	G	B	B	B	G	B	G
25 Pubescence Calyx Tube	F	F	F	+	F	F	+	F	F	F	F	F
26 Node of 1st Flower	4-5	4-5	4-5	5-6	4-5	4-5	6	7	5-6	8-9	5-6	6-7
27 Time of 1st Flower	MID-AUGUST					LATE AUGUST						
28 SEED — Colour	Pp	W	Pp	Pp	Pp	Pp	Pp	W	Pp	B	Pp	Pp
29 SEEDLING — Hypocotyl Colour	++	-	++	++	++	+	++	++	++	-	++	

E.M. and M. groups

Character	BACCHUS MARSH	BURNLEY	MADRID	MILTON	HORSHAM	HILL'S SMALL	SAMARIA	YEA	EDENHOPE	MT. BARKER	WHITE SEEDED MT. B.	AMBER SEEDED MT. B.	RED LEAF	SMEATON
1 LEAF COLOUR of LAMINA — Grey Green														
2 Mid-Dark Green	+	+	+	+	+	+	+	+	+	+	+	+	-	+
3 PATTERN of LAMINA — Pale Central Mark	C1	C3 5	C3 5	C1	C1	C2	C1	C1	C2- 1	C2	C2	C2	-	C3 4
4 Anthocyanin Upper Sur.	A7 B	A7	A2 7	A7	A7	A5	A5	A7 5	A5 7	A5	-	-	A1	A6
5 Anthocyanin Lower Sur.	A7 B	-	A9 7	-	-	-	A7	A6 7	A7	-	-	-	A1	A6
6 HAIRINESS — Upper Surface	+	F	+	+	+	+	+	F	+	+	+	+	-	+
7 Lower Surface	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8 *SIZE — Diameter of Leaflet	L	L	L	M	S	M	M-L	M	M	M	M	M	M	M
9 PETIOLE — *Length	L	L	L	M	M	L	M	M	L	M	M	M	M	M
10 Colour	Bg	Bg	Bg	Bg	B	Gb	Gb	Gb	B	B	G	G	Bg	B
11 Hairiness	+	F	F	+	+	+	+	F	+	+	+	+	+	F
12 STIPULE — Colour	-	S2	S3	-	-	S3	S2	S2	S2	S2 3	-	-	S3	S3
13 Pubescence	+	+	+	+	F	+	+	+	+	+	+	+	+	+
14 *INTERNODE — Length above 4th Flower	L	L	L	L	M	M-L	M	M	M-L	M-L	M-L	M-L	M-L	L
15 *LATERALS — Number per Runner	3-4	4-5	3-5	6	4	5-7	4	5	5-7	5	3-5	3-5	3	4
16 Degree of Branching	1-2	1-2	1	1	1	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1	1
17 *RUNNER — Number per plant	M	M	M	M	M	M	M	M	M	M	M	M	M	M-L
18 STEM — Colour	Bg	Gb	Bg	Bg	B	Gb	Bg	B	Gb	B	G	G	G	B
19 Pubescence	+	-	-	+	+	+	+	F	+	+	+	+	+	+
20 Thickness	M			S						M				
21 FLOWER COLOUR — Corolla Bud	W	W	W	W	W	W	W	W	W	W	[W]	[W]	P	W
22 Open Corolla	W	W	W	W	W	W	W	W	W	W	[W]	[W]	PT	W
23 Calyx Tube	-	-	-	-	-	K2	K2-3	K3	K3	K2	-	-	K3	K2
24 Calyx Lobes	BG	G	B	G	BG	G	G	G	GB	G	G	G	G	G
25 Pubescence Calyx Tube	F	F	F	F	F	F	F	F	F	F	F	F	F	F
26 Node of 1st Flower	7-8	8-9	9	9	9	9	B-9	8-9	9-10	9-11	11	10	10	12
27 Time of 1st Flower	EARLY SEPTEMBER							MID-SEPTEMBER						
28 SEED — Colour	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	W	B	Pp	Pp
29 SEEDLING — Hypocotyl Colour	+	+	+	+		+	+	+	+	+	-	-	+	+

L.M. group

Character	CASTERTON	HEXHAM	MANSFIELD	DERRINAL	BENALLA	BERLIN	NANGEELA	KYABRAM	BURNERANG	PAHANTANU	MERINO	MACARTHUR	RUAKURA SELn
1 LEAF COLOUR of LAMINA — Grey Green													
2 Mid-Dark Green	+	+	+	+	+	+	+	+	+	+	+	+	+
3 PATTERN of LAMINA — Pale Central Mark	C3 5	C1	C2 1	C3 5	C1	C1 2	C3 4	C1	C2 3	C2 3	C2	C3	C3 5
4 Anthocyanin Upper Sur.	A5 7	A7	A3 B	A7	A2 7	A7	A7	A5 7	A7	A2 7	A7	AB	A7
5 Anthocyanin Lower Sur.	A6 7	A7	A6	A7	A7	A6	A6 7	A6	A6	A7	A7	-	-
6 HAIRINESS — Upper Surface	+	+	+	+	+	F	+	+	+	F	F	+	+
7 Lower Surface	+	+	+	+	+	+	+	+	+	+	+	+	+
8 *SIZE — Diameter of Leaflet	L	M	M	M	M	S	L	M	M	L	M	M	M
9 PETIOLE — *Length	M	M	M	M	M	M	M	M	S	M	M	M	M
10 Colour	B	G	Gb	B	B	B	G	G	G	B	G	B	-
11 Hairiness	+	+	+	F	+	+	+	+	+	+	+	+	F
12 STIPULE — Colour	S3	S3	S3	S2	S3	S3	S̄3	S2	S3	S3	S3	S3	S3
13 Pubescence	+	+	+	F	+	+	+	+	+	+	+	F	-
14 *INTERNODE — Length above 4th Flower	M	M	M-L	M	M	M	L	M	S	M	M	M	M
15 *LATERALS — Number per Runner	7	7	12	L	6	9	8-12	11	12	7	6-7	10	L
16 Degree of Branching	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	2-3	1-2	1-2	1-2	1-2
17 *RUNNER — Number per plant	M	M	M-L	L	M	M	M-L	M-L	M	L	L	L	M
18 STEM — Colour	B	Bg	Bg	Gb	B	Gb	G	B	G	Gb	Gb	Gb	G
19 Pubescence	+	+	F	+	+	+	+	+	+	F	+	+	+
20 Thickness											M-L	M	
21 FLOWER COLOUR — Corolla Bud	W	W	W	W	W	W	W	W	W	W	W	W	W
22 Open Corolla	W	W	W	W	W	W	W	W	W	W	W	W	W
23 Calyx Tube	-	K3	K3	-	K3	K3 4	K3	K3	K3̄	K3	K3	K3	K3
24 Calyx Lobes	G	G	G	G	G	B	GB	G	G	G	G	G	G
25 Pubescence Calyx Tube	F	F	F	-	F	-	F	F	F	F	-	-	
26 Node of 1st Flower	11	11	14	13	11 12	11 12	13 14	15	13	12 13	13	13	15
27 Time of 1st Flower	LATE SEPTEMBER										EARLY		
28 SEED — Colour	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp
29 SEEDLING — Hypocotyl Colour	+	+	+	+	+	+	+	+	+	+	+	+	+

L. group

Character	TALLAROOK	WENIGUP	ROSTOCK	FLINDERS	WODONGA	ROJEN	PHILIP ISLAND	KYNETON	BASS	BASS WHITE SEEDED	RUAKURA FARM
1 LEAF COLOUR of LAMINA — Grey Green											
2 Mid-Dark Green	+	+	+	+	+	+	+	+	+	+	+
3 PATTERN of LAMINA — Pale Central Mark	C3 5	-	C1	C1 2	C1	C1	C1 2	C1	C2	C2	C1
4 Anthocyanin Upper Sur.	A2 7	A7	A2	A5 7	A2 7	A2 7	A2 7	A2 7	A2 7	-	A2 7
5 Anthocyanin Lower Sur.	A6 7	A7	A7 9	A7	A6 7	A6 7	A7	A6 7	A6 7	-	-
6 HAIRINESS — Upper Surface	+	-	F	F	F	F	F	+	F	F	F
7 Lower Surface	+	+	+	+	+	+	+	+	+	+	+
8 *SIZE — Diameter of Leaflet	M	M-L	L	S-M	M	M	S-M	S-M	M	M	M
9 PETIOLE — *Length	M	M	M	M	M	S	M	M	S	S	M
10 Colour	B	G	B	B	Gb	Gb	Gb	G	Bg	G	B
11 Hairiness	F	F	-	+	F	+	+	+	+	+	F
12 STIPULE — Colour	-	-	S3	-	-	S3	S3	S3	S2 3	-	S2 3
13 Pubescence	F	F	-	F	F	+	F	F	F	F	F
14 *INTERNODE — Length above 4th Flower	M	L	L	M	M	M	M	M	M	M	M
15 *LATERALS — Number per Runner	12	M	L	10	12	11	15-16	14	B-9	8-9	11
16 Degree of Branching	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
17 *RUNNER — Number per plant	L	M	M-L	L	L	L	L	L	L	L	L
18 STEM — Colour	Gb	Gb	B	Gb	G	Bg	G	G	Bg	G	Gb
19 Pubescence	+	-	-	+	+	+	+	+	F	F	+
20 Thickness						M-L	M	M	L		
21 FLOWER COLOUR — Corolla Bud	W	W	W	W	W	W	W	W	W	[W]	W
22 Open Corolla	W	W	W	W	W	W	W	W	W	[W]	W
23 Calyx Tube	-	-	-	K3	-	K3̄	K2 3	K3	-	-	K3
24 Calyx Lobes	GB	G	G	BG	G	GB	G	GB	G	G	G
25 Pubescence Calyx Tube	-	-	-	-	-	-	-	-	-	-	F
26 Node of 1st Flower	16 17	15 16	16 17	16	16	16 17	17 1B	15 16	17 19	17 19	1B 19
27 Time of 1st Flower	MID-OCTOBER						LATE				
28 SEED — Colour	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	Pp	W	Pp
29 SEEDLING — Hypocotyl Colour	+	+	+	+	+	+	+	+	+	-	+

TABLE 2A.

LEAF.	STEM.	FLOWER.
Hairiness of Lamina—	*Length of Internode above 4th inflorescence of basal runner—*	*Corolla—*
— .. No Hairs	S. .. ½ inch	[W] .. White
F .. Few Hairs	M .. 1 inch	W .. White with pink veins
+ .. Hairy	L .. 2 inches +	P .. Pink
+ + .. Very Hairy		P.T. .. Pink Throat
		R .. Red
	Number of Runners per Plant—	
Fourth Leaf from Tip of Basal Runner—	S .. 5–11	
Size—Diameter of leaflet—	M .. 12–18	
S .. 1 cm.	L .. 19–25	
M .. 1½ cm.		SEED.
L .. 2¼ cm.	*Stem Colour—*	
Length of Petiole—	B .. Brown	*Seed Colour—*
S .. 2 inches	G .. Green	W .. White
M .. 3–5 inches		B .. Brown
L .. 6 inches		Pp .. Purple-black
	Stem Pubescence as for Leaf—	
	* .. Appressed hairs	
	Stem Thickness—	
	S .. Slender	
	M .. Medium	
	L .. Stout	

TABLE 3.—VARIATIONS RECORDED FOR *T. subterraneum* IN EUROPE AND AUSTRALIA.

—	Hairiness.			Stipule Pink Striped.	Leaf Pale Mark.	Leaf Dark Flecks.	Corolla Coloured.	Calyx Red Bands.	Node First Flower.	Time of Flowering.	Number of Varieties.
	Plant.	Leaf.	Calyx.								
ENGLAND.											
Curtis—London	+	+	..	+	..	+	–	+	2–3	June–Aug.	..
Smith	+	+	..	– +	..	..	..	..	..	..	..
Fitch and Smith	+	..	..	..	..	..	..	..	..	..	..
Hooker	++	+	..	..	..	..	–	..	1–2 ?	May–June	..
Trimen and Dyer—Middlesex ..	+	..	..	..	..	..	..	..	..	..	..
Bentham and Hooker	+	..	..	..	..	..	..	..	..	..	..
Avebury	+	..	..	..	..	..	–	..	2–3 ?	..	..
Sowerby and Smith	+	++	..	+	– +(white)	..	–	..	4–7	..	..
Sowerby and Syme	+	++	..	+	– +	..	–	..	4–7	..	..
Pratt	+	..	..	–	..	..	–	+	4–6 ?	May–June	..
Ray	..	..	..	..	..	..	–	..	..	..	..
Rees	..	+	..	+	..	..	..	..	..	..	..
Withering	+	+	..	..	..	..	–	..	..	..	..
Hooker and Arnott	+	++	..	..	..	..	–	..	..	..	..
J. Hill, Flore—Britain, 1760 ..	+	+	..	..	..	..	..	..	2–3 ?	..	..
Davies, W.—J Min. Ag., 1927 ..	+	+	..	..	..	– +	–	– +	..	..	..
FRANCE.											
Coste	+	..	..	..	..	..	–	..	..	..	..
Cosson and St. Pierre	++	++	+	..	..	..	–	..	..	May–July	..
Cosson and Germain Paris ..	..	++	..	..	..	..	..	..	..	..	..
Lamarck	+	..	..	..	..	..	–	..	..	..	..
Bonnier—N. France	+	..	..	..	..	..	..	..	..	..	..
Grenier and Godron W.F. Mid. F.	+	..	..	..	..	+ –	–	..	..	..	..
Spach—North France	+	..	..	..	..	..	–	..	..	..	..
Rouy and Foucard	+	..	..	..	..	..	–	–	..	..	..
GERMANY.											
Huth	+	+	..	..	..	..	..	–	1–2	..	..
Reichenbech	– +	..	+	–	..	..	–	–	6–7 ?	..	..
Hegi—Mid. Europe	+	..	..	..	..	..	–	..	..	..	..
SOUTHERN EUROPE.											
Boissier—Fl. Orient	+, ++	..	..	..	..	..	–	..	..	..	..
Linnaeus—France, Italy ..	+	..	..	..	..	..	..	..	..	..	..
Ascherson and Graebner ..	+, ++	+, ++	– +	..	..	..	+ –	+ –	..	..	4
De Halacsy—Greece	+	..	–	..	..	..	..	..	..	..	..
Willkomm and Lance—Spain ..	+, ++	..	..	–	..	..	–	..	..	..	..
Cocconi and Parma—Spain ..	+	..	+	..	..	..	..	..	..	..	..
Lazaro E. Ibiza—Spain ..	..	..	..	..	..	..	–	..	..	..	..
Fiori and Paoletti—Italy ..	+	..	..	..	..	..	–	– +	..	..	3
Bertolinii—Italy	+	..	..	–	+	..	–	–	..	..	..
Gay—Algeria	..	..	..	..	..	..	–	–	..	..	..
Post—Palestine	+	..	..	..	..	..	+ –	..	..	..	2
Ullman	+, ++	+, ++	..	..	..	..	+ –	+ –	..	..	5

TABLE 3.—VARIATIONS RECORDED FOR *T. subterranean* IN EUROPE AND AUSTRALIA—*continued.*

—	Hairiness. Plant.	Leaf.	Calyx.	Stipule Pink Striped.	Leaf Pale Mark.	Leaf Dark Flecks.	Corolla Coloured.	Calyx Red Bands.	Node First Flower.	Time of Flowering.	Number of Varieties.
SOUTH AUSTRALIA.											
Black, 1909	+	..	..	..	..	..	−	+	..	..	..
„ 1922	..	..	..	..	..	..	−	− +	..	Oct.–Dec.	..
WESTERN AUSTRALIA.											
Adams, A. B., 1927	+ +	+	..	..	− +	− +	−	− +	..	Aug.–Nov.	4
„ 1929	..	..	..	..	..	..	+ +	..	..	..	5
„ 1930	..	..	..	..	..	+ +	..	..	..	..	6
„ 1932	..	..	..	..	..	..	..	+ +	..	..	7
„ 1934	..	..	..	..	..	..	..	..	..	..	9
„ 1938	..	..	..	..	..	..	..	..	..	..	10
TASMANIA.											
Radel, 1935	+ +	+	..	−, +, + +	−, +, + +	−, +, + +	−	−, +, + +	..	Sept.–Oct.	9 (from Vic., S.A. and W.A.)
SOUTH AUSTRALIA.											
Donald and Smith, 1937 ..	..	..	..	..	..	..	..	..	..	Aug.–Oct.	14 (from Vic., S.A. and W.A.)
NEW ZEALAND.											
Levy and Gorman, 1937 ..	..	..	..	− +	..	..	..	− +	..	Aug.–Oct.	15 (from Vic., S.A. W.A.)
VICTORIA.											
Harrison, 1936	+	..	..	−	+ (white)	..	−	−	15	Oct.	1 Late
Drake, 1940	+ +	..	..	−	+	+	−	−	8	Sept.	1 E. Mid.
Aitken and Drake, 1940 ..	−, +, + +	−, +, + +	− +	−, +, + +	−, +, + +	−, +, + +	−, +, + +	−, +, + +	4–20	Aug.–Oct.	50 V. Early—V. Late.

the hairs on the upper lamina result in a greyish green appearance of the leaf (Dwalganup, Springhurst, Pinkflower). Other varieties are less densely hairy with mid-green leaves (Mt. Barker, Bacchus Marsh), while others have glabrous upper leaf surfaces, having some hairs or being completely without hairs on petiole, stem and stipule (Reigert's White, Wenigup, Burnley) (cf. Plate XIX., figs. 3–6). The presence of hairs on the lower surface of the leaf lamina is the only degree of pubescence held in common by all the varieties.

Origin of Varieties.

The list of varieties in Table 2, includes all the available ones isolated so far in Australia, and Appendix I. lists the areas from which each type (except Mt. Barker) has been obtained from 1929 onwards. So far the only indication of any particular area of concentration of varieties (cf. Vavilov (24)) is the preponderance of Victorian varieties in Table 2, but this is probably due to the more intensive search for them in this State. While there is no definite evidence to show that any or all of the varieties as they exist to-day were introduced accidentally from imported seeds or other material, there is also no evidence for an Australian origin of major variations.

Considering the varietal characters in two groups, (*a*) time of flowering (regarded as an expression of length of vegetative period, and capacity for branching), and (*b*) hairiness, anthocynanin, and leaf pattern, etc., only variations in respect to the first group are likely to be selected by the environment in a change of climate, such as from England to Southern Australia (Forster and Vasey (12)).

Combining with the Australian collection, the few varieties of direct European origin examined, one finds almost exactly the same range of maturity groups from both sources. Of the six European samples, those from Rouen and Berlin provided plants of both early and late maturity, while the others were pure lines in every respect; "Madrid" and "Liege" being early and similar to the early types from Rouen and Berlin, and "Cambrai" and "Rostock" being of the one late variety, distinct from the late Rouen and Berlin types.

No European variety exactly resembles any variety in the Australian collection, but the differences are only in "minor" and not in "major" characters, and if only the major characters are considered, counterparts can be found for each of the four European types in the Australian collection. The late variety "Rouen" is very close to "Kyneton" (Victoria); the early variety "Madrid" is much less hairy than the Victorian variety "Bacchus Marsh" of the same maturity group, and differs in leaf markings; the variety "Rostock" is like the W.A. variety "Wenigup" in growth form and maturity, but differs in flower and leaf characters.

A survey of all available European descriptions and illustrations of the species (Table 3) shows few records of variations in minor characters and in node of first flower, and none of variations in leafiness. Only in 1934 did Ullmann (23) emphasize the practicability of selection of various maturity types, after a close study of Australian literature. He also described five European varieties (Appendix 2) varying in relative length of peduncle and petiole, in flower, calyx colour and in hairiness. The description of variety "longipes" fits our variety "Wenigup" and that of variety "brachycladum" is very similar to the variety "Second Northam." It is interesting to note in Table 3 that a form with a red banded calyx and striped stipules predominates in the English references, while a form with a green calyx and stipule is the one most described in French, German, Italian and Spanish records. Williams (27) found at Aberystwyth that the "Australian variety" (presumably Mt. Barker) had less anthocyanin flecking and colouring compared with the local native variety, but only a test under standard conditions, of samples from various English districts, could indicate the identity of the several red calyxed varieties with any in England, or with the variety "brachycladum" recorded in Italy (Appendix 2). Similarly, a much more extensive collection should be made in Europe and tested against the local varieties with green calyces, before their introduction here from abroad could be proved.

Mutations may have occurred in Australia in such characters as anthocyanin distribution, though the only direct evidence so far is the discovery of the white-seeded "bud" mutation in Dwalganup quoted above.

Evidence as to the origin of maturity types is difficult to obtain. The commonly accepted explanation of the development of district or local ecotypes by the operation of environment on a population of mixed genetic constitution, can be applied to any of the normally cross-pollinated pasture plants, as any sample will show marked plant to plant variation. This process cannot apply with equal force to a self-fertilized plant like *T. subterraneum* in any variety of which there is a remarkable lack of variation from plant to plant, in characters which might allow of environmental selection or modification.

There is no evidence whatever of any heritable response to altered environment in any of the varieties grown at Burnley over a period, in some cases, of twelve years. Seed has been harvested from each variety and resown the next year, but the varieties have shown an extreme stability of type, with no alteration of flowering date or other major characters, under an environment often widely different from that of the district where collected. A survey of the natural occurrence of maturity types in Victoria (fig. 7) shows some correlation of maturity type with length of growing season, as would be expected from Trumble's emphasis on the minimum length of growing season necessary for effective seed production, and regeneration of the early and midseason varieties (21). Examination of fig. 7 shows a preponderance of the early varieties in unirrigated areas with about seven months growing season, and of the late varieties in the districts of South Gippsland, with nine to twelve months growing season. The data used in this map for the length of growing season usual in Victoria, is part of that published in 1939 by Trumble (22). The occurrence of two areas in which the winter temperature becomes too low for growth, should be noted, and also the areas south of Swan Hill which have the warmest winter in the State.

Late varieties, even if introduced, are unable to seed and persist in short season districts, but an early variety is able to persist if introduced into a district which would support a later strain, especially if not subject to competition from a later strain. As is shown in fig. 7, many early and mid-season varieties have been obtained from areas which could support a later strain and in some cases do so as well as the earlier one. Thus the natural occurrence of a certain maturity type in any given locality depends on (*a*) the chance of its arrival, (*b*) the local growing season as controlled by climate and topography, being at least the minimum necessary for regeneration, and (*c*) its ability to compete with other varieties or species present.

A late variety, on account of its capacity for greater seed production, will tend to dominate an earlier strain in a district of long growing season, but several maturity types often occur either separately or in mixture in the same locality, e.g. at Smeaton, " Bacchus Marsh," " Smeaton " and " Mount Barker "; at Drouin, " Bass," " Mt. Barker " and " Dwalganup "; at Ballan, " Bacchus Marsh," " Mt. Barker " and a late type; at Tumbarumba, " Mt. Barker " and " Tallarook ;" while around Benalla, early, mid-season, late mid-season and late varieties occur. In a district of short climatic growing season, such local environments as river flats or irrigated fields allow the predominance of much later types, e.g. " Tallarook " near Seymour and a late volunteer type at Rochester, and in low areas near Yea, Tylden and Ballarat, where the mid-season " Mt. Barker " or earlier varieties otherwise predominate.

The whole of the varietal distribution data suggest a chance distribution, with environment preventing the persistence of late types in short-season districts, and more effective regeneration (heavier setting of seed) favouring the dominance of late varieties in long-season districts. In intermediate districts, several maturity types may co-exist, although in competition there is usually a tendency for a particular type to dominate in any one position.

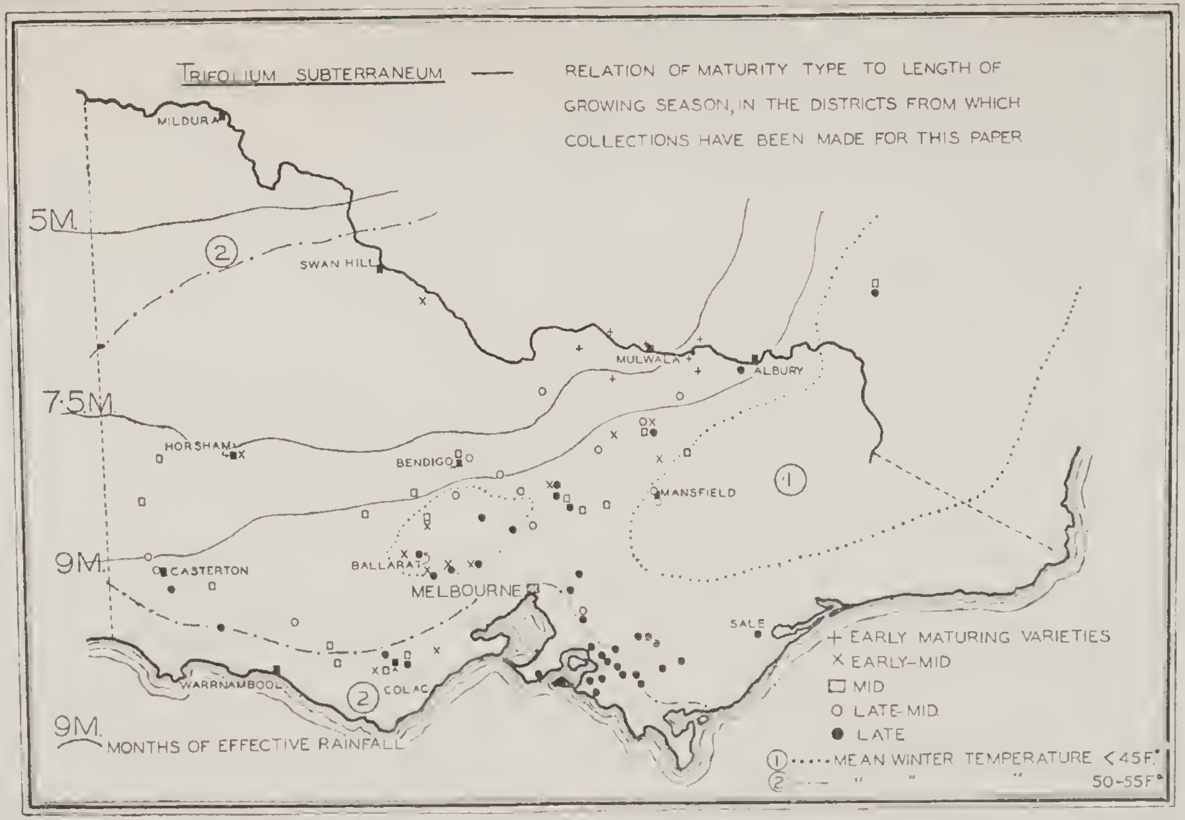

Fig. 7.—Map of Victoria, showing the relation of maturity type to the length of growing season, in districts from which collections have been made for this paper.

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With no evidence of the occurrence of major variations in Australia, the evidence pointing to the accidental introduction into Australia of many of the varieties as they exist to-day, is summarized as follows:

1. The non-occurrence of any heritable alteration in major characters of the varieties when grown at Burnley over a period of twelve years, under changed environment.

2. The relatively short history of the species in Australia considered in relation to the extreme range of types isolated.

3. The absence of any closer correlation between the distribution of maturity types and the length of growing season of the district of their occurrence than can be explained as above (fig. 7 and discussion).

4. The existence in Europe of varieties differing only in minor, but not in major characters from certain Australian varieties.

This evidence appears to preclude the view that the varieties represent local ecotypes, developed in response to environment. The important question of how and when maturity variations occur may be answered only by close study under controlled conditions of the present maturity types, in order to find whether heritable variations in maturity can be induced.

Relation of Time of Sowing to Growth and Reproduction.

As an essential to the elucidation of the biology of *T. subterraneum* and to its best use as a pasture plant, the influence of time of sowing on its growth and reproduction has been investigated.

Sowings were made at weekly or fortnightly intervals at the Agricultural School and at Burnley Gardens, between May 1936 and April 1940. Three varieties were selected for study, as being typical of three major groups of Subterranean Clover, differing in the flowering date when sown in Autumn. The early variety "Dwalganup," flowering in early spring, and the late variety, "Tallarook," flowering in late spring, were studied from September 1938 onwards. The mid-season variety, "Mt. Barker," flowering in mid-spring, was used from May 1936; all sowings were made from stocks of certified seed.

In addition, in 1938 and 1939, simultaneous sowings of stock seeds from nine varieties, were made at several centres in Victoria and Tasmania, and in 1940, at several centres in New South Wales. In 1938, 1939 and 1940, the photoperiodic response was studied on certain plots from May to September. Each sowing consisted of at least fifty plants, sometimes many more. They were sown at 3-inch intervals with about a foot between the rows. In all cases, the seeds were sown with superphosphate and the plants watered when necessary, so that germination was never inhibited by lack of moisture in any of those tests.

Observations were made on the dates of (*a*) brairding—the appearance of cotyledons above ground; the period from sowing to brairding, being the *brairding period;* (*b*) flower initiation—the appearance of the first flower primordium at the tip of a basal runner; this was found by dissection under a binocular microscope (fig. 2), the period from brairding to flower initiation being the *rosette period*; (*c*) first flowering—this was taken as the date when the first flowers had appeared on half the number of plants in the row of 50; the *flower development period* is from initiation to first flowering; (*d*) seed formation—i.e. when seeds have developed in the burr sufficiently to be capable of germination; the *period of ripening* covers the time from the opening of the first flower to its successful seed formation. "*The vegetative period*" is that from sowing to first flowering.

The accuracy of the observations on the stages of development was high during most of the year, and variations in spacing had no effect on the time of flowering. As in wheat (12), the flowering dates recorded for plants of the summer sowings were variable, being somewhat affected by disease—Rust (*Uromyces trifolii*), a type of mosaic, and Red spider (*Tetranychus*), but much more so by variations in the physiological response of the varieties at that season.

A. Effect of Time of Sowing on Development.

As will be shown in the succeeding sections of the paper, the time of sowing had relatively little effect on the time taken for the periods of brairding, and ripening, compared with its effect on flowering, which was primarily an effect on flower initiation. The influence of time of sowing varied greatly with the variety.

Table 4.—Showing data for time required for germination of soft seeds of three varieties of *T. subterraneum*.

Incubator.		Field.		
Temperature °C.	Days to Production of Free, Green Cotyledons.	*	Soil Temperature at three inches Depth—Range of Fortnightly Means.	Time of Year.
30	2·9	..	..	..
25	3·1	6	20–28	Nov.–Feb.
20	4	..	..	..
15	6	10	17–19	April
10	9	17	10	July
5	22	..	..	..

* In the Field Tests, the seed was planted at about 3/8ths of an inch.

(1) *Brairding.*—In all varieties, the brairding period varied from six days (November to February) to ten days (early April), and to seventeen days (early July). It seems clear that this range could be reasonably attributed to soil temperature alone, as previous laboratory experiments had shown that in no variety did light affect the rate of germination of the seed. Table (4) compares the time taken, under various temperatures, for germination of the seed to the stage of free, green cotyledons, under laboratory and field conditions. The variation in the time taken for brairding in the field is of the same order as that taken under equivalent laboratory temperatures, but is relatively slower. This difference is more apparent than real, and is due to the impractibility of obtaining exactly comparable stages and conditions in the field and in a laboratory.

(2) *Flowering.*—During 1937 and 1938, observations were made on dates of seeding, brairding and flowering, and in 1939 and 1940 on the date of flower initiation also. Data for 1938 and 1939 have been arranged in fig. 8 to show the relation between time of sowing, and flowering, in the three varieties. Less than the number of observations are shown in these and other graphs for the sake of clarity, but no aberrant cases have been omitted.

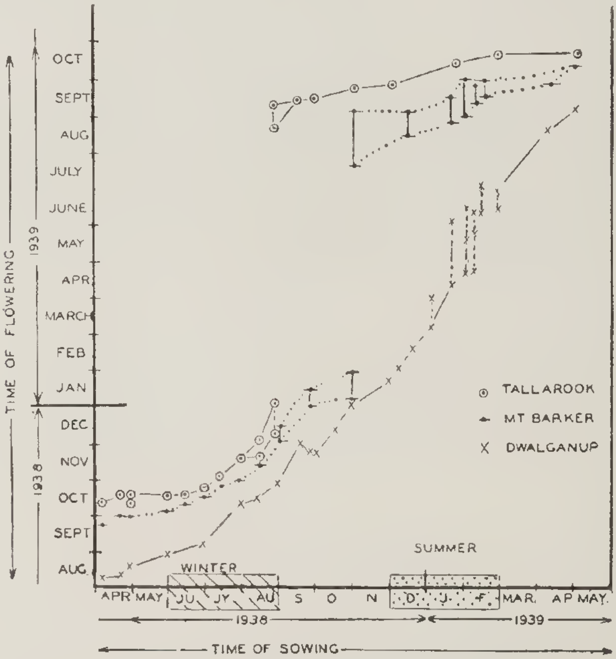

FIG. 8.—Graph showing the effect of time of sowing on time of flowering in three varieties in 1938 and 1939.

In the " Tallarook " variety, the plants sown at the usual time in early autumn (April), flowered in early October, after a vegetative period of six months. Sowing up to July reduced the vegetative period, but the date of flowering remained the same. Plants of the mid-August sowing, showed some variation in time and node of flowering (shown in the graph as a vertical line between the dates of first and last plants to flower). Some individuals were as much as four weeks later than others, instead of the normal difference of about one week. The late August sowings gave a few plants with runners which began flowering in December, but then died back, though the rest of the plant, and other plants of the row remained vegetative till the end of August 1939. From mid-August to late August is termed the " critical period " of sowing, because of the resultant variable flowering dates. The sowings from September onwards, produced plants that began flowering from the next September. Thus, there was a period of eight months vegetative growth between the flowering of the August sowings and that of the September ones. This behaviour recalls that of winter wheats sown in summer. (See fig. 2, Forster and Vasey (12).)

The results for " Mt. Barker," are comparable with those for " Tallarook," but certain differences are obvious from fig. 8. Attention is drawn to the fact that the critical period ends two months later, and, on the whole, the vegetative period is shorter for " Mt. Barker," whatever the time of sowing.

The behaviour of the " Dwalganup " variety showed marked differences from the other two. Plants sown in early April, flowered in August, a month before " Mt. Barker," and the date of flowering became progressively later with successive sowings. The critical period was from mid-January to the end of February, there was no period of complete cessation of flowering. During this period occasional plants entirely failed to flower and those which did develop, were confined to one or two runners. Sometimes there was a difference of two to three months between the flowering of runners on the same plant. The runners that flowered, varied in position from basal to younger ones, but once a runner started to flower, it proceeded normally.

It is noteworthy that the sowing of mid and late varieties during Summer, results in the plants flowering at about the same time as those sown in Autumn. This has also been noted from observations on self sown swards at Burnley, and at the Werribee Research Farm, where plants of mid and late season varieties, which had germinated in January, flowered in the same week as those sown in April (see also 6, 8, 10, 16).

In fig. 9, the vegetative periods for the three varieties are plotted against their respective times of sowing for 1937-40. It will be seen that the time of sowing has a great effect on the

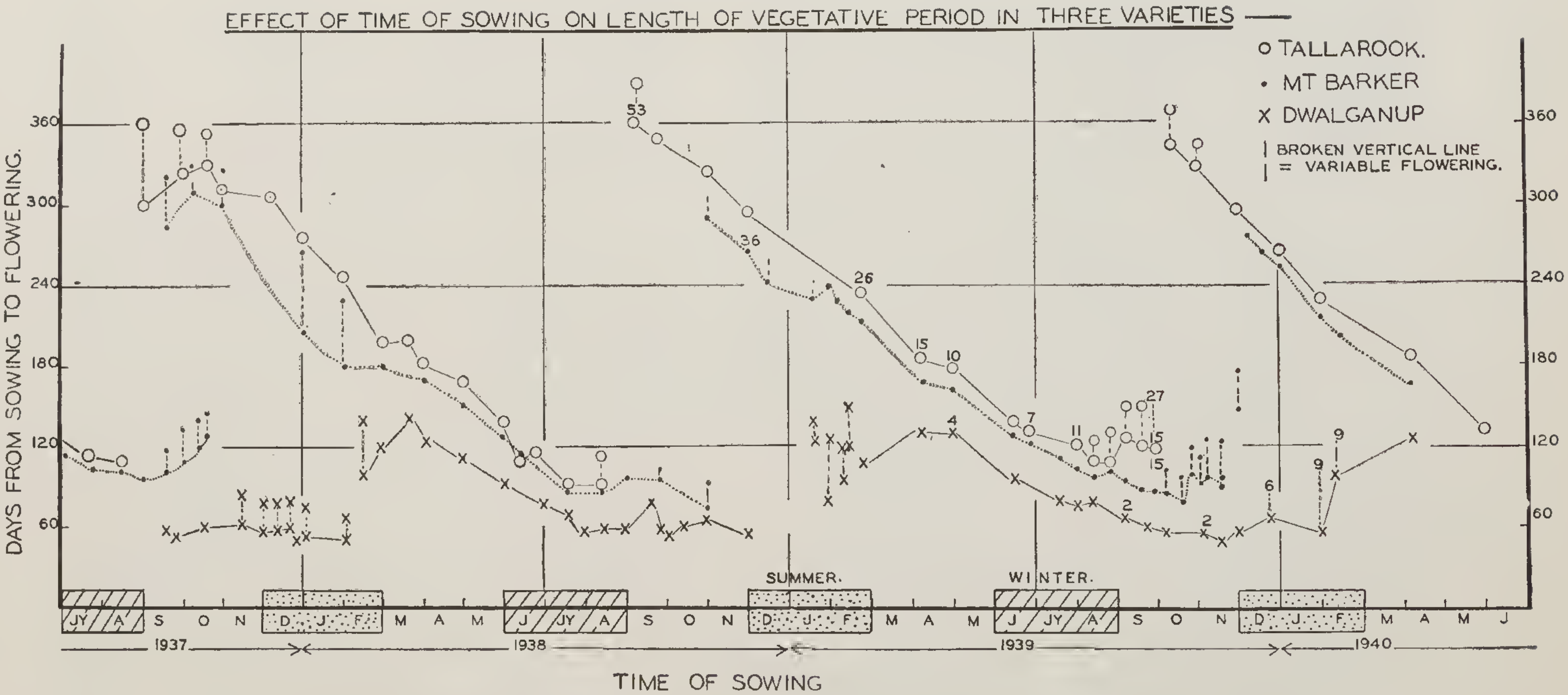

Fig. 9. Graph showing the length of vegetative period in relation to time of sowing in three varieties, 1937 to 1940.

length of the vegetative period, which diminishes as sowings progress from Summer to the next Spring. The magnitude of this effect is summarized in Table 5.

TABLE 5.—EFFECT OF TIME OF SOWING ON LENGTH OF VEGETATIVE PERIOD, IN THREE VARIETIES OF EARLY, MID, AND LATE MATURITY. (Sowing from September, 1938, to August, 1939.)

Variety.	Longest Vegetative Period.		Normal Vegetative Period.		Shortest Vegetative Period.	
	Months.	Time of Sowing.	Months.	Time of Sowing.	Months.	Time of Sowing.
"Dwalganup" (early)	4	April–May	4	Early April	$1\frac{3}{4}$	September to January
"Mt. Barker" (mid.)	9	Late November	5	Early April	$2\frac{3}{4}$	Early October
"Tallarook" (late)	12	Early September	6	Early April	$3\frac{1}{3}$	August

The problem is seen to be more complex, when the position of the flower on the basal runners of the plant, is examined. In fig. 9, for the 1939 observations, the node at which the first flower was produced, is indicated at several locations on the graphs. Immediately after the critical period in each variety, when the period of vegetative growth is at its longest, the number of the node at which the first flower is produced is highest. For the three varieties, the numbers of the nodes at which the first flowers form, from these sowings, are 50, 36 and 4 respectively. In plants originating from sowings immediately before the critical period, the corresponding figs. were 7, 6 and 2 respectively. Plants originating from the critical period of sowing, show an increasing variation between individual runners.

The season has some influence on the onset of the critical period, as is shown by a comparison of the years 1937 and 1938, with 1939. This will be discussed later.

(3) *Flower Initiation.*—It was of some interest to discover whether the variability in flowering was due to an effect on flower primordia formation, or on flower development. This was investigated for the three varieties in 1939 and 1940. The results for "Mt. Barker" are set out in fig. 10, which shows that the primary influence is on primordia formation. Fig. 11 shows, for all three varieties, the length of the interval between primordia formation and flowering, and also in many cases, the number of the node at which the first flower appeared. It seems reasonable to assume that the changes in the length of the flower development period from month to month, are explicable as an effect of temperature on the rate of growth of the primordia.

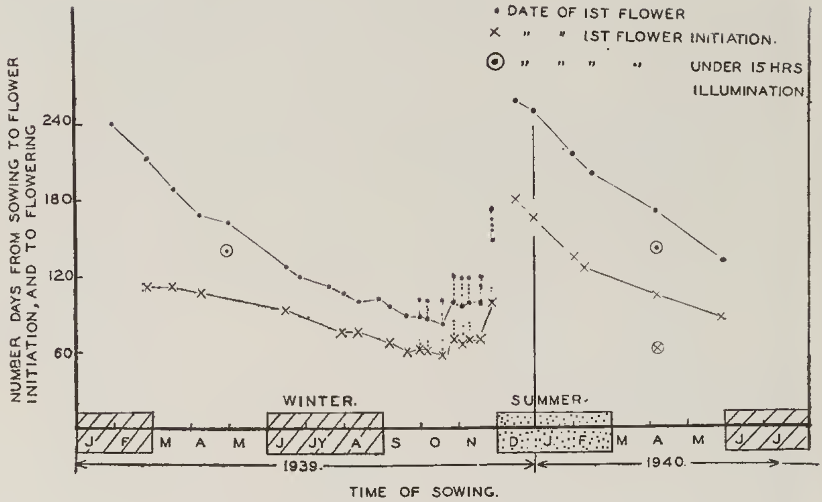

FIG. 10.—Graph showing the length of time from sowing to flower initiation, and to flowering, and hence the relation between flower initiation and flowering in "Mt. Barker" variety.

Such a temperature effect was observed in the rate of node formation along a runner; the rate increased from mid-winter to the summer months, being fourteen days per node in winter, and only four days in the summer.

Fig. 11 also indicates the time at which flower initiation begins. For "Tallarook," the earliest flower initiation began in mid-August, and continued normally till the beginning of November;

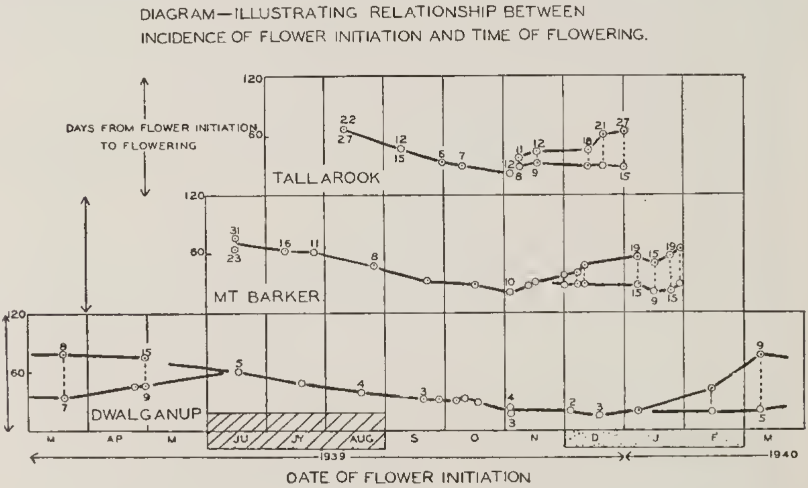

FIG. 11.—Graph showing the relation of the length of the period from flower initiation to flowering, to the date of flower initiation; and the incidence during the year of the beginning, variability and end of initiation for the three varieties.

for " Mt. Barker " the corresponding period was from mid-June to the beginning of January; and for " Dwalganup," flower initiation occurred all through the year, but was somewhat unstable between February and March in 1940, and March and April in 1939.

(4) *Seed-formation.*—The time taken for seed-formation, varied from one month after flowering in October, to about three months, after flowering in May. Exposure to a fifteen hour length of day in the winter did not hasten the period, and it is apparently dependent on the temperature only. The developing embryo may be killed a few days after flowering, through desiccation in summer, or by low temperatures in winter.

A wide variation in size of seeds often occurs along a runner, due to the time interval between the flowering of the first and the last axillary inflorescences along a runner, and to the decreasingly favorable conditions for seed development during this period.

Comparing the April sown varieties at Burnley, the minimum time taken for seed formation varies from about six weeks for the early varieties, to five weeks for the mid-season ones and to four weeks for the late ones. For the main maturity types, the total period necessary from sowing to seed formation, for minimum regeneration is thus assessable, and can be compared with the average length of growing season of any district.

B. Discussion.

It is obvious from the preceding sections, that the time of sowing has a marked effect upon the length of the vegetative period, and on the time of flower initiation and flowering. (The less marked effects on brairding and flowering will not be discussed here.) There is ample evidence in other work, that these effects might be due to the differences in length of day, to which the plants from the various sowings were subjected during their development. An experiment on Subterranean clover itself, has shown that a continuous, long period of daily illumination may cause earlier flowering. Certain plants were treated from May to September, with an extra period of artificial illumination the intensity of which was 30-foot candles at plant level. The total period of continuous illumination was fifteen hours in each twenty-four. This treatment, applied to plants of each variety, sown in April and in February, caused the flowers to appear three or more weeks earlier than on the controls. As might be expected, the plants whose vegetative period had been reduced by the " long day," produced flowers on nodes nearer the axis, i.e., these plants resembled morphologically, field plants produced by sowings just before the critical period. Thus while the node number of the first flower in " Dwalganup," was reduced from four to two, in those of "Mt. Barker" it was reduced from ten to six, and in those of "Tallarook," from fifteen to seven (Plate XVII., fig. 1, and cf. fig. 9). It is concluded that the later the variety, the greater the response to a summer length of day, in respect to the "node number."

Examination of fig. 12 will show however, that the interpretation of the field data is by no means as simple as is suggested by these experiments on the effect of increased daily illumination. The variations in the length of day experienced throughout the year in Melbourne, are plotted as a heavy line on the upper section of fig. 12; the mean minimum weekly temperatures from September, 1938, to December, 1940, are shown as a fine line. In the lower section, length of vegetative period, flower initiation, and flowering during this period, are arranged horizontally, in the order of sowing. The data is for "Tallarook."

There is some indication that the length of day may affect the date of flower initiation in the field. Thus at *E*, the time from sowing to initiation is five months, and the length of day ranges from 9½ to 11 hours. As the length of day increases, the time from sowing to initiation decreases, partly due to an increased rate of growth, but also, to initiation at a lower "node-number"; e.g. at *A*, where the node-number is only seven, compared with that of fifteen resulting from the sowing at *E*.

Comparing the group of sowings from *A* to *B* however, it can be seen that they all have about the same vegetative period. But the plants sown at *A*, had an average length of day of 10½ hours, and flower initiation began before it reached 12½ hours per day, whereas those sown at *B* germinated when the length of day was 12½ hours, and began flower initiation when it reached 15 hours per day. Intermediate sowings gave intermediate results. Here, obviously, the average length of day during the growing period is not correlated with the time of initiation or the length of vegetative period. Comparing those plants sown at *C*, and at *D*, it is clear that they are both subjected to approximately the same length of day (rising from 11 to 14½ hours per day), during the first two months of their vegetative life. Those at *C* formed flower primordia in early November 1938 (two months after sowing), but those at *D* failed to form primordia till August 1939 although experiencing a length of day of 14 to nearly 15 hours from November to February. Thus it is clear that some factor or factors, in addition to day length, must affect flower formation, at any rate during Spring.

The incidence of high temperatures was then analysed, as it had been shown experimentally by Hamner and Bonner (14), and Roberts and Struckmeyer (20), that night temperatures above a certain level prevented flowering in a number of plants, including some legumes, despite a favorable length of day. The

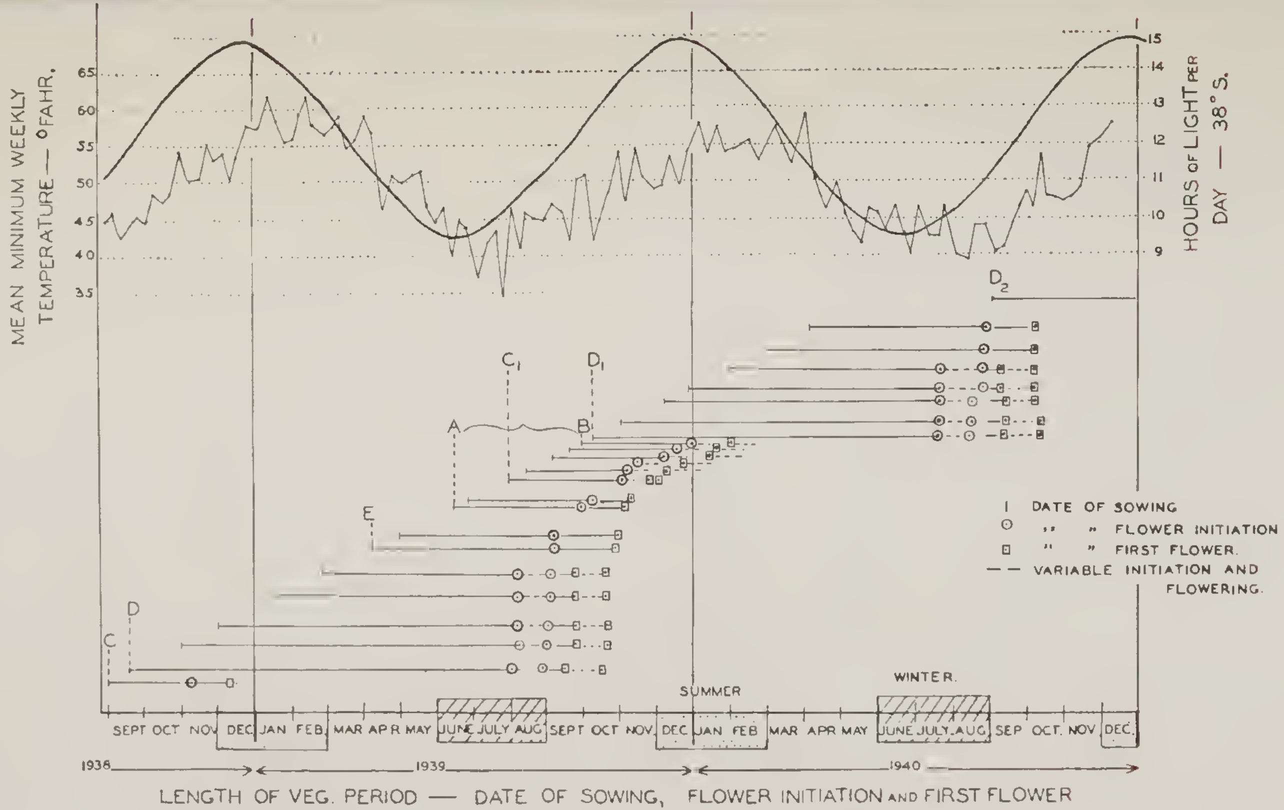

FIG. 12.—Graph, showing the relation between length of day and mean minimum temperature, and the behaviour of plants sown in the years 1938 to 1940.

figures for the mean minimum weekly air temperatures are therefore given also in fig. 12. It is evident that the temperature rises and falls with the length of day, but the maxima and minima occur about a month later in each case.

Referring again to the sowings at *C* and *D*; the plants sown at *C* developed under a night temperature increasing from 45°F. to 50°F. But the temperature only rose above 50°F. the week before flower initiation, and it fell to 50°F. again, for the next two weeks, before rising above this level continuously for the next four months. Those sown at *D*, however, experienced a rise in temperature to above 50°F. several weeks before flower initiation would have occurred, judging from the time taken in plants of the previous sowing. The remarkable difference in the flowering dates for *C* and *D* is shown in fig. 12.

Considering the behaviour of plants sown in the spring of 1939, at D_1 temperatures above 50°F. became continuous two months after sowing, and again flower initiation did not occur till the next July.

Plants sown from C_1 to *B*, correspond to those of the previous year, sown at *C*. That is, they are not subjected to continuous periods above 50°F., and they have a short vegetative period. These plants showed an increasing variability of flower initiation, and it can be seen that flower initiation in this group occurs over the perod when the temperatures were fluctuating about 50°F.

Considering the results in both years, the first sowing resulting in a prolonged vegetative period, occurred earlier in the spring of 1938, than in that of 1939; it is clear that the temperature level rose continuously above 50°F., earlier in 1938.

It is therefore concluded provisionally, that the failure to flower, in plants sown just after the critical period, is possibly due to the incidence of high temperatures. If the weekly minimum temperature rarely falls below 50°F. at a time when flower primordia could normally be formed, then it seems that even under the most favorable length of day, flower initiation is inhibited. If this explanation is correct, then it follows (see fig. 12) that this temperature effect is not rapidly reversible, for even when the temperature has fallen well below 50°F., the plants sown at D_1 still remain vegetative for four months. They finally form primordia, when the temperature is between 40° and 45°F., and the length of day only $10\frac{1}{2}$ hours.

There is, however, another possible explanation of the prolonged vegetative period in the plants of type *D*. It may be that flowering is delayed not because of a high temperature but because of the lack of a suitably low temperature. In the normal vernalization theory, as applied to wheats, the low temperature "thermo-stage" must precede the "photostage." Applying this to clover we note that plants sown between *A* and *B* pass the early stages of their vegetative life when the temperature is between 35° and 45°F. and they flower after a relatively brief vegetative period. Plants sown at D_1, however, experience a high temperature (as well as a long day) during their early vegetative life and do not form primordia until late winter, when the temperature has again fallen to between 40° and 45°F. The evidence therefore allows the suggestion that the plant must receive at some time in its vegetative period, and not necessarily before it receives the long day, a period of low temperature before flower initiation can take place.

If the view is adopted that flowering is inhibited by high temperatures, then the data indicate that the inhibition temperature is near 50°F. for "Tallarook," 53°F. for "Mt. Barker" and about 58°F. for "Dwalganup." A temperature level sufficient to prevent flower initiation in "Dwalganup" is not reached in Melbourne, but the critical period of flowering in this variety is associated with fluctuations about the 58°F. level. If we adopt however the vernalization hypothesis (i.e. necessity for a low thermo-stage) then it may be that "Dwalganup" differs from the other two varieties in not requiring temperatures below 45 C. during its thermo stage. Only further experiments can distinguish between the two hypotheses, and these are now being undertaken.

C. Effect of Latitude and Season on Time of Flowering in Autumn Sown Plants.

It has been shown that with autumn sown plants, the later the variety the greater the response to a long daily period of illumination; and that the earliest flower initiation possible in "Tallarook" varied between the end of July to the middle of August, with a length of day increasing from 10 to 11 hours. Conversely, the earliest variety could initiate flower primordia all through the year, though it could be hastened slightly by lengthened daily illumination. It might thus be reasoned that the late variety grown in more northerly districts in southern Australia, would show earlier flowering due to the earlier incidence of a length of day of more than 10 hours, and conversely with districts south of Melbourne. To investigate this effect of latitude, sowings of nine varieties of early to late maturity were made at places ranging from Launceston to Sydney.

Typical results of such sowings are shown in Table 6, but only those for the varieties "Dwalganup," "Mt. Barker" and "Tallarook" are included as they are representative of the early, mid and late maturity groups.

Considering the varieties sown in early April of 1938, those at Walpeup flowered about a week earlier than those at Burnley, but of those at Launceston, the late variety flowered at the same

TABLE 6.—EFFECT OF DISTRICT OF SOWING ON THE TIME OF FLOWERING OF VARIETIES, WHEN SOWN AT THE SAME TIME.

Places Where Tested.	Latitude.	1938.			1939.			1940.			Incidence 11-hour Day in Spring.	10-hour Day.
		Dwalganup.	Mt. Barker.	Tallarook.	Dwalganup.	Mt. Barker.	Tallarook.	Dwalganup.	Mt. Barker.	Tallarook.		
Sown April 8.												
Sydney	33° ·5	..	..	..	..	..	..	July 25	Sept. 4	Sept. 25	Aug. 19	July 6
Canberra	35° ·2	..	..	..	..	..	..	Aug. 28	Oct. 5	Oct. 23	,, 20	,, 12
Walpeup	35° ·1	Aug. 10	Sept. 19	Oct. 4	Aug. 12	Sept. 25	Oct. 5	,, 17	Sept. 21	,, 7	..	..
Rutherglen	36°	,, 12	,, 25	,, 6	,, 12	,, 27	,, 9	..	..	..	..	..
Burnley	38°	,, 15	,, 27	,, 12	,, 17	,, 27	,, 24	,, 14	,, 22	,, 5	,, 24	,, 7
Launceston	41° ·2	Sept. 5	Oct. 3	,, 11	Sept. 7	Oct. 10	,, 24	..	..	..	,, 28	Aug. 1
Sown April 28.							Sown April 29 Kerang (35° ·4)					
Swan Hill	35° ·2	Aug. 20	Sept. 21	Sept. 25	..	..		Aug. 25	Sept. 25	Oct. 12	..	..
Cohuna	35° ·4	,, 16	,, 21	Oct. 3	..	..	..	..	..	..	..	..
Burnley	38°	Sept. 1	Oct. 1	,, 13	..	..	..	..	..	..	..	..
Launceston	41° ·2	,, 23	,, 7	,, 14	..	..	..	..	..	..	..	..

time as that at Burnley; the mid-season one was a week later, and the early one was three weeks later. At Walpeup in 1939, only "Tallarook" flowered much earlier than at Burnley, while at Launceston the late variety again flowered at the same time as at Burnley. Taking a length of day of 11 hours as probably necessary for flower initiation in "Tallarook" plants sown in autumn, it can be seen in Table 6 that this is reached by Aug. 24, at Melbourne, and only four days earlier and later respectively, at Walpeup and Launceston. The difference is comparatively small, and also, no clear gradient is obvious in the commencement of flowering of the late variety in the three places. The difference between the results of the earlier varieties at Melbourne and Launceston is much greater than between Melbourne and Walpeup, and the later flowering of "Tallarook" in 1939, compared with 1938 at both Burnley and Launceston, is also noteworthy, as occurring when a cooler spring period was experienced over these districts. In addition, the node at which the first flower was produced along the basal runners, did not vary in number, as would have occurred with a sufficiently wide difference in incidence of the favorable length of day. It may be reasonably concluded that differences in temperature levels experienced in the various districts, affect the rate of vegetative growth of the varieties, and so their times of flowering. This is further supported by the observation that the plants reached certain stages in their growth (such as that of four leaves per plant) earlier, when growing in the northern districts, and later, when growing at Launceston; and that flower initiation in "Tallarook" was some days earlier at Walpeup and Kerang, than with plants sown at the same time in Melbourne.

In 1940, results were also obtained from Sydney and Canberra. Comparing them with those from Walpeup and Burnley, it is interesting to note that the first three places have approximately the same incidence of an 11-hour day, but the flowering of all three varieties is much earlier at Sydney, and much later at Canberra than at Walpeup. Reference to such a measure of temperature levels as the average monthly minimum temperature, shows that the figures for Sydney, from June to September are from 7° to 4°F. above those for Melbourne, while for Canberra, they are from 9° to 7°F. less than those for Melbourne.

Within the range in length of daily illumination occurring from Launceston to Sydney, the effect of decreased latitude for the plant appears to be due to the associated trend in climate from cool to warmer, and where two districts of the same latitude differ sufficiently in the temperature levels of the growing season, the varieties will show the differences, in their commencement of flowering.

Observations on Production According to Variety and Environment.

Most of the following notes on the comparative productive capacity of varieties have been taken on the spaced plants in the plots at Burnley Gardens. Growth under such conditions gives any plant the chance to show its full productive capacity, and also allows comparative study of varietal development; whereas under sward conditions, lateral development, functioning leaves, number of runners, and hence differences between varieties, are much reduced. These observations are thus preliminary to the essential investigation of varietal production in relation to sward conditions and to length of growing season. They are, however, free from the effects of the factors of competition, which may act differentially between varieties.

From two to four typically developed plants were used for each measurement. The quantitative data obtained from these observations were scarcely likely to be statistically acceptable, as the basis of detailed hypotheses, but they were sufficient to give information on varietal differences of a major type.

Time of varietal differentiation in leaf, dryweight, and burr production.

To ensure that the varieties could be compared in their development, observations were taken on those plots in which they had all germinated in the second week in April.

It has been pointed out previously, that many of the varieties differ greatly from each other in leaf and dryweight production at flowering time, owing to variations in runner development. A periodic count of leaves per plant in 1940, the results of which are set out in Table 7, showed that this variation commenced in late June, after which date, initiation of new runners was slower in the early variety, "Dwalganup," and the late one,

Table 7.—Comparative leaf development in varieties at various dates—1940.

Maturity.	Variety.	Number of Leaves per Plant at—					
		July 1.	Aug. 1.	Aug. 22.	Sept. 17.	Oct. 15.	Nov. 15.
V.E. ..	Dwalganup ..	15–20	20–30	15–25	40	120	120
V.E. ..	Mulwala	20	30–40	25–30	100	200	250
E. ..	Reigert's White ..	20	20–30	30–40	60	..	230
E. ..	Springhurst ..	25	30	25–30	40	200	220
E.M. ..	Bacchus Marsh ..	25	30	35–40	100	450	550
M. ..	Mount Barker ..	25	30	30	60	430	450
L.M. ..	Nangeela ..	25	30	30–40	90	650	650
L.M. ..	Mansfield ..	25	40–60	40–60	130	750	750
L.M. ..	Burnerang ..	25	40–60	40–60	140	400	670
L. ..	Merino	20	30–40	40	..	500	750
L. ..	Macarthur ..	20	40–50	40–50	120	500	820
L. ..	Tallarook ..	15	40–50	40–50	70	550	850
L. ..	Bass	15	30	30	80	500	750
L. ..	Wenigup ..	10	15–20	20	40	..	400

"Wenigup," than in the other varieties; hence the former varieties showed fewer leaves. By August, the late mid-season varieties, "Mansfield" and "Burnerang," had developed twice, and the late ones, "Macarthur" and "Tallarook," nearly twice the number of leaves formed by some of the early varieties; by mid-September, "Mansfield," "Burnerang" and "Macarthur" had more than three times the leaves of "Wenigup" and "Dwalganup," owing to their larger numbers of runners and laterals; but in this particular year, relative figures were not quite normal because of the unusually dry period. By November, most of the later varieties had six or more times the number of leaves of the now senescent earliest ones. Consequently, the ratio of leaf to dryweight was much higher in the leafy later varieties.

It is clear from Table 7, that the rate of leaf production per plant was small up to September, and that the general great increase in growth responsible for the well known "spring flush" in the field, began in mid-September, under Melbourne conditions. Such an increase had been noted about the same time in 1938, and some weeks later, in 1939. Reference to fig. 13, giving the figures for mean soil temperature at 6 inches,

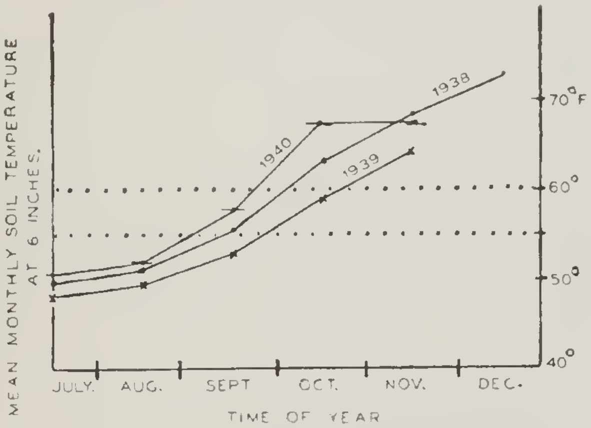

FIG. 13.—Graph, showing the incidence of the rise in mean soil temperature at 6 inches, in the spring of 1938, 1939 and 1940, at Melbourne.

between July and December, showed that in the three years, the conspicuous increase of growth occurred when the temperature rose from below 55°F. to above 60°F. Thus the incidence of the spring flush may be expected to vary according to the district, because of its particular characteristics of spring temperature levels. Early spring feed thus depends on the possibility of getting a useful increase in leaf production earlier than usual—earlier in September for Melbourne. A closer study of varietal growth during this time should show if any variety has outstanding value in this direction.

The dryweight per plant increases with plant development and with the "maturity type" of variety, but varieties within each "maturity type" group differ somewhat in this respect. Table 8 shows that in mid-September, there is little difference in dry-weight between the varieties, but by early November, the range between them is wide, and of the two with very early maturity, Mulwala is distinctly superior.

TABLE 8.—INCREASE IN DRY WEIGHT AND BURRS PER PLANT WITH MATURITY, 1937.

Maturity.	Variety.	Date— September 15. Dry Weight. (*a*)	September 15. Burrs. (*b*)	November 1. Dry Weight. (*a*)	November 1. Burrs. (*b*)	December 16. Dry. Weight. (*a*)	December 16. Burrs. (*b*)
		Grams.	No.	Grams.	No.	Grams.	No.
V.E. ..	Dwalganup ..	5	10	8	70	7·5	60
V.E. ..	Mulwala	7	4	29	40	35	100
E. ..	Daliak	3	5	9	130	..	..
E. ..	Springhurst ..	4·6	0	25	60	27	100
E.M. ..	Bacchus Marsh ..	4·2	0	33	85	35	340
M. ..	Mount Barker ..	5·3	0	24	16	35	450
L.M. ..	Nangeela	6	0	48	6	..	..
L.M. ..	Burnerang ..	6	0	27	4	50	370
L. ..	Tallarook	5·2	0	26	8	60	550
L. ..	Bass	5·6	0	..	0	45	220
L. ..	Wenigup	6	0	22	2	70	50

The time at which seed production begins, varies according to the time of maturity of the variety. Table 8 shows that the early varieties had developed several mature seeds by late September, and had finished seed production by November; the mid-season varieties had begun in mid-October and finished in late November, while the corresponding periods for the late ones were in early November and late December. The early cessation of seed formation in the early varieties, in addition to their relatively small capacity for flower production, causes a lower amount of seed formation than in the later maturing varieties. The time taken from germination to the formation of the first burr, gives an indication of the minimum length of growing season necessary for the minimum regeneration of the variety.

EFFECT OF GROWING SEASON ON VARIETAL PRODUCTION.

Investigations by Donald and Smith (10) at the Waite Institute 1935-36 showed that leaf and dryweight production was closely correlated with lateness of maturity, as measured by the time of flowering. The lengths of the growing seasons in the two years of their experiments were 5.5 and 5.0 months respectively owing to late sowing, although the full growing season during these particular years was 8.7 and 7.6 months. They also demonstrated the importance for seed-setting and maturation, of the daily evaporation from the eighth to the twenty-eighth day after commencement of flowering, and the superiority of "Tallarook" in setting seed under severe conditions. Their investigations were repeated under Melbourne conditions, in order to find the relevance of these results locally. In 1937 it was observed that the date of the appearance of the greatest number of flowers per plant—"maximum flowering"—occurred from four to three weeks later than the opening of the first inflorescence, and this is probably connected with the significance of the evaporation level during the first month after flowering.

Eleven varieties were selected to represent a full range of maturity groups, and several other varieties gave additional data. Observations were taken at the end of the growing seasons of 1937-8-9 on seed-setting, and on the dryweight per plant, excluding roots and burrs (see Table 9).

The plants were sown in the first week of April in the three years during which observations were taken. Seven of the varieties were among those which had been tested at Adelaide. The same order of result was obtained except that the increased number of late varieties studied, showed that the seed-setting of Tallarook was not particularly superior, even under drought conditions.

In 1937, the growing season was somewhat dry (7.6 + months) for Melbourne, and the length of growing season ($^{P}/_{E} > \frac{1}{3}$) beginning from April, was 7.3 months. The dry-weight per plant showed an increase in relation to maturity (col. *a*), in the few varieties tested. The yield of burrs per plant (col. *c*), also increased with length of the vegetation period of the type, but within the early group, the variety "Mulwala" developed more burrs than another early one, "Dwalganup", and among the late varieties, "Tallarook" gave a higher yield than "Wenigup." However, considering the yield of seeds per burr (col. *d*) it is seen that there is comparatively little variation between the varieties, and that if "Wenigup" is excepted as a variety with abnormal inflorescence formation, there is no indication of a lower yield in the later varieties. This lack of agreement with the Adelaide results was thought to be due to the lower evaporation prevailing in late spring. In this particular season, Melbourne weather conditions (6.3 + months) suited the late varieties.

In 1938, a drought year, the growing season was only 5.3 months ($^{P}/_{E} > \frac{1}{3}$) from April, and the spring evaporation was very close to that experienced in Adelaide in 1936. The dry-weight per plant again showed an increase with lateness, but as a result of the dry conditions, the weight per variety was reduced, especially in the later ones, compared with the yield in 1937. The yield of burrs (*c*) was variable, but "Bacchus Marsh" and

TABLE 9.—A COMPARISON OF SEED-SETTING AND DRY WEIGHT PRODUCTION PER PLANT IN THE GROWING SEASONS OF 1937-39, IN VARIETIES RANGING IN MATURITY FROM EARLY TO LATE.

Maturity.	Variety.	1937.			1938.						1939.					
		Dry Weight per Plant (exc. burrs or roots).	Burrs per Plant.	Seeds per Burr.		Inflorescences per Plant.			Seeds per Inflorescence.	Ratio of Burrs to Inflorescenses						
		a.	c.	d.	a.	b.	c.	d.	e.	f.	a.	b.	c.	d.	e.	f.
V.E.	Dwalganup	7·5	60	..	7	165	25	2·6	0·4	1 : 6·5	10	170	50	2·8	0·9	1 : 3·2
V.E.	Mulwala	15	100	2·8	10	285	80	2·7	0·7	1 : 4	25	460	240	2·6	1·4	1 : 1·9
E.	Springhurst	21	100	..	17	295	50	2·6	0·4	1 : 6	25	300	90	3·2	0·9	1 : 3·3
E.M.	Bacchus Marsh ..	32	340	2·8	22	595	125	3·3	0·7	1 : 4·8	55	900	450	3·0	1·5	1 : 2
M.	Mt. Barker	..	420	2·6	10	340	45	2·4	0·3	1 : 8	30	1330	590	2·6	1·2	1 : 2·2
L.M.	Mansfield	..	..	..	30	930	165	2·3	0·4	1 : 6	..	..	..	..	..	..
L.M.	Nangeela	..	..	3·2	34	570	30	2·4	0·1	1 : 21	50	1130	670	3·0	2·3	1 : 1·7
L.M.	Burnerang	50	380	2·7	19	195	80	2·0	0·3	1 : 2·5	30	1250	860	2·8	1·2	1 : 1·5
L.	Macarthur	..	..	2·7	21	540	65	2·6	0·3	1 : 8	..	1580	1500	2·9	2·8	1 : 1·05
L.	Merino	..	..	..	29	675	60	..	..	1 : 8	..	900	..	2·5	..	..
L.	Tallarook	60	550	3·2	27	635	95	2·6	0·37	1 : 7	50	1350	1030	2·8	2·0	1 : 1·3
L.	Wenigup	70	220	2·1	25	730	41	2·2	0·1	1 : 1·8	..	550	150	3·3	0·9	1 : 3·7
L.	Bass	..	..	2·8	..	..	..	..	..	..	..	2060	1010	4·0	2·0	1 : 2

"Mansfield" gave the best yields. Compared with that of the previous year, there was a definite indication of the yield of later varieties being more reduced. However, the yield of seeds per burr (*d*) showed only a slight decrease in most cases. Further data obtained (cols. *d, e, f*), gave more information. A count of the approximate number of inflorescences (col. *d*), produced per plant, showed a general increase with lateness of maturity, but there were large differences between varieties of the one group, e.g. "Burnerang," "Naugeela" and "Mansfield." The Melbourne figures for seeds per inflorescence (which in Adelaide were associated with evaporation), showed that several varieties were poor, compared with the rest, e.g. "Naugeela," in the late midseason group, and "Wenigup," in the late group; but there was again no definite trend of low yield with lateness. The varieties "Mulwala," "Bacchus Marsh," "Mansfield" and "Tallarook," appeared somewhat superior to the rest under drought conditions. The figures for the ratio of number of burrs to number of inflorescences (col. *f*) emphasizes the inferior burr-formation of "Naugeela" and "Wenigup."

In this particular season the climatic conditions for Melbourne being similar to the seasons investigated by the Adelaide workers, gave similar results; but the variety "Tallarook" is now seen to have its equals in other late varieties, "Merino" and "Macarthur."

In 1939, a year of abnormally high rainfall, the growing season from April was 8.3 months. All the columns show an increase in yield compared with 1938, especially in cols. *e* and *f*. The increase is particularly noticeable in the varieties that gave the poorest yields under drought conditions.

The effect of the 1938 and 1939 growing seasons was also shown by the yields of burrs from sample quadrats taken on an established sward of the "Mt. Barker" variety. In 1938, the yield from a decimetre square quadrat was 120 burrs, and in 1939, 250 burrs with more than twice the total number of seeds.

From the data on varietal flowering and seed production at Melbourne, a tentative table (Table 10) has been drawn up, to indicate the length of growing season necessary for minimum and aggressive regeneration of some of the more important varieties. The figures stated by Trumble for three varieties, are included, and are necessarily somewhat lower because of the higher temperature level available in South Australia for growth in the winter.

It is noteworthy that in 1938, with a growing season of only 5.3 months, ending just before September, both the mid and the late varieties set a fair amount of seed, under spaced plot conditions. Even under sward conditions "Mt. Barker" set as much seed as in the variety plot. It seems likely that, at least in Melbourne, plants can continue growth sufficiently to set seed, for at least two months after the ratio of precipitation to evaporation has fallen below one-third. This should be tested further in order to define more accurately the effective length of growing season for Southern Victoria.

TABLE 10.—MONTHS OF EFFECTIVE RAINFALL, FOR PERSISTENCE OF VARIETIES IN SOUTHERN VICTORIA, WHEN GERMINATING SECOND WEEK IN APRIL.

Maturity.	Variety.	Months to First Flower.	Months to Minimum Seed-setting.	Months to Maximum Seed-setting.	Months for Persistence in South Australia (4, 15).
V.E. ..	Dwalganup ..	4·5	6·0	7·5	6
V.E. ..	Mulwala	4·5	6·0	7·5	..
E. ..	Springhurst ..	4·8	6·0	7·5	..
E.M. ..	Bacchus Marsh ..	5·1	6·5	7·8	..
M. ..	Mt. Barker ..	5·5	7·0	8·0	7·5
L.M. ..	Mansfield	5·7	7·0	8·5	..
L. ..	Macarthur ..	5·9	7·0	8·5	..
L. ..	Tallarook	6·2	7·0	8·5 +	8·25
L. ..	Bass	6·5	7·5	8·5 +	..

Summary.

The type variety of the species in Australia—" Mt. Barker," is fully described.

Genotypical variation is found to occur in the same characters as occur in such other leguminous species as *Vicia sativa,* and *Pisum sativum.* Fifty varieties are described by means of a table of observed heritable characters.

The characters are grouped as major—those influencing the growth structure, and so the plant's capacity to produce leaves, flowers and seed, and minor—those causing variations in anthocyanin development in leaf, calyx, corolla, stipule, stem, seed and hypocotyl, and hairiness of plant surfaces (leaf, stem, and calyx).

Five major characters make up the " basal runner organization," which is typical for each variety. The characters of number of runners per plant, lateral development, internode length, and seed-production per plant, are all strongly influenced by the time of flowering peculiar to each variety. But, in addition to this influence of time of flowering on productive capacity, there is variation in these major characters within each maturity group, so that choice is possible of the most productive variety for a given length of growing season.

There is indirect evidence that the range of maturity types has not originated under various local conditions within the last 50 years, though there has been some control of the predominance of the early and late maturity types in Victoria, through length of growing season. There is some evidence that minor mutations, changing the anthocyanin development characteristic of a variety, have occurred in Australia.

The time of sowing through the year, influences the length of the vegetative and ripening periods, in the three varieties studied in detail. The time taken for brairding, for flower development, and for ripening, varies in the same order for the three varieties, because of variations in rate of growth due to temperature. The time taken for the rosette period, varies, not only with the rate of growth at a given season, but also according to the variety, and particularly with lateness of variety.

The later the variety, the greater the response of the plant in respect to a lowering of the "node-number," to a summer length of day, applied experimentally. In the field, however, owing to the effect of high temperatures, only for a short period in spring does the increasing length of day hasten flower initiation.

It was found that, in the late variety "Tallarook," flower initiation becomes variable, and then fails, when the minimum weekly temperatures rise above 50°F., and thus the vegetative period suddenly lengthens. The temperature level falls below this in April, but initiation is still prevented, and it is only in late July or early August that plants sown at any time in the previous mid-spring and summer form flower primordia. This failure to commence flower initiation under high temperatures, results in a much longer vegetative period than if variations in rate of growth were the only cause; under conditions preventing summer drought, several months extra grazing can thus be obtained from summer sowings after the critical date.

This may be due, either to a direct repressive effect of high temperature on flower initiation, with a consequent after-effect lasting several months after the temperature has fallen below "critical" level, or to the indirect effect of high temperature, owing to the necessity for a period of low temperature, as a prelude to the formation of flower primordia.

The mid-season variety—"Mt. Barker"—is comparable, except that the critical temperature level is about 53°F., and consequently the first sowing to result in a prolonged vegetative period, is later, and flower initiation is somewhat earlier in the following year—mid-June.

In the early variety, "Dwalganup," sowing throughout the year resulted in flowering all through, but a period of variable initiation occurs from January to March, associated with temperatures fluctuating above a level of about 67°F.

In the three varieties, the higher the number of the node at which the first flower is produced, compared with the typical number resulting from an early autumn sowing, the greater the degree of repression of initiation that has occurred.

Varieties sown at the same time, in localities ranging from Launceston to Sydney, showed variations in times of flowering related to the winter-spring temperature level of the locality; the late varieties were the least affected.

In observations on seed-production per plant, in relation to a dry season, certain varieties were superior in each maturity group, and there was no trend of reduced seed setting per inflorescence with lateness.

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APPENDIX I.—DISTRICTS FROM WHICH THE LISTED VARIETIES OF *T. subterraneum* HAVE BEEN OBTAINED.

Variety.	Maturity.	Locality of Origin.
Dwalganup	E. 1	Boyup Brook, Western Australia
Dwalganup White Seeded	E. 2	Dwalganup, Western Australia
Muresk	E. 3	Muresk, Western Australia
Second Northam	E. 4	Northam, Western Australia
Mulwala	E. 5	Mulwala, Corowa, Coreen, New South Wales; Quat Quatta, Victoria
Daliak	E 6	Western Australia
Northam	E. 7	Northam, Western Australia
Reigert's White	E. 8	Yarloop, Western Australia
Pink Flowered	E. 9	Muresk, Western Australia
Yabba North	E 10	Yabba North, Victoria
Springhurst	E 11	Springhurst, Victoria
Baulkamaugh	E.	Baulkamaugh North, Victoria
Seaton Park	E.M. 1	Seaton Park, South Australia
Bacchus Marsh	E.M. 2	Myrniong, Smeaton, Myall, Windermere, Victoria
Burnley	E.M. 3	Burnley, Victoria
Madrid	E.M. 4	Madrid, Spain; Leige, France
Milton	E.M. 5	Milton, New South Wales; Seymour, Sebastopol, Winchelsea, Irrewillipe East, Victoria
Horsham	E.M. 6	Horsham, Victoria
Hill's Small	E.M. 7	Cobden, Mount Noorat, Irrewillipe East, Victoria
Samaria	E.M. 8	Samaria, Benalla, Violet Town, Victoria
Yea	M. 1	Yea, Koriella, Victoria

APPENDIX I.—*continued.*

Variety.	Maturity.	Locality of Origin.
Edenhope	M. 2	Edenhope, Goroke, Whitfield, Victoria
Mount Barker	M. 3	Mount Barker, South Australia
Mount Barker White Seeded	M. 4	Mount Barker, South Australia
Mount Barker Amber Seeded	M. 5	Mount Barker, South Australia
Red-leaf	M. 6	Upper Lurg, Victoria
Smeaton	M. 7	Smeaton, Victoria
Casterton	L.M. 1	Casterton, Bendigo, Victoria
Hexham	L.M. 2	Hexham, Nalinga, Casterton, Victoria
Mansfield	L.M. 3	Mansfield, Wangaratta, Docker's Plains, Kyabram, Delatite, Toolborac, Benalla, Victoria
Derrinal	L.M. 4	Derrinal, Victoria
Benalla	L.M. 5	Benalla, Kyabram, Victoria
Berlin	L.M. 6	Berlin, Germany
Nangeela	L.M. 7	Nangeela, Bendigo, Victoria
Kyabram	L.M. 8	Kyabram, Victoria
Burnerang	L.M. 9	Wangaratta, Victoria
Pahautanui	L.M. 10	Pahautanui, New Zealand
Merino	L. 1	Merino, Victoria
Macarthur	L. 2	Macarthur, Victoria ; Bothwell, Tasmania
Ruakura Seln.	L. 3	Ruakura, New Zealand
Tallarook	L. 4	Tallarook, Seymour, Bena, Korumburra, Romsey, Warncoort, Carisbrook, Warrion, Victoria ; Tumbarumba, New South Wales
Wenigup	L. 5	Bridgetown, Western Australia
Rostock	L. 6	Rostock, Cambrai, Europe
Flinders	L. 7	Flinders, Victoria
Wodonga	L. 8	Wodonga, Victoria
Rouen	L. 9	Rouen, France ; Berlin, Germany
Phillip Island	L. 10	Phillip Island, Monomeith, Victoria
Kyneton	L. 11	Kyneton, Victoria
Bass	L. 12	Bass, Beaconsfield, Tooradin, Mooroolbark, Yering, Glen Alvie, Woolamai, Yannathan, Loch, Monomeith, Caldermeade, Warragul, Bena, Leongatha, Victoria
White Seeded Bass	L. 13	Burnley, Victoria
Ruakura Farm	L. 14	New Zealand

APPENDIX II.—KEY TO VARIETIES OF *T. subterraneum* (ADAPTED FROM ULLMANN.)

A. PETIOLES SHORT TO MEDIUM-STALKED.

1. Peduncle little longer or shorter than subtending petiole—

(*a*) Runner mostly 10–15 cm. long, peduncle about as long as subtending petiole, head with 2–5 florets, burr the size of small hazelnut. The most usual form. Variety *genuinum* Rouy, *typicum* Asch. & Gr.

(*b*) Plant small, runner mostly 3–8 cm. long; plant strongly hairy, leaves with felt like hairs; peduncle much shorter than petiole or almost absent; head with 2–3 florets; burr the size of a pea; calyx often sparsely softly hairy, mostly red coloured; sterile florets with shorter calyx teeth. Occasional in dry, stony places. Variety *brachycladum* Gib. & Belli (2nd Northam) ?

2. Peduncle all or mostly much longer than subtending petiole. Plant lax, elongated, runner mostly 25–40 cm. peduncle 4 times longer than petiole; stipules long, pointed; head with 3–5 florets; burr, the size of small hazelnut; corolla not noticeably veined. Only in Southern Medit. Districts. Variety *longipes* Gay. (Wenigup ?)

B. PETIOLES OVER 10 CM. LONG.

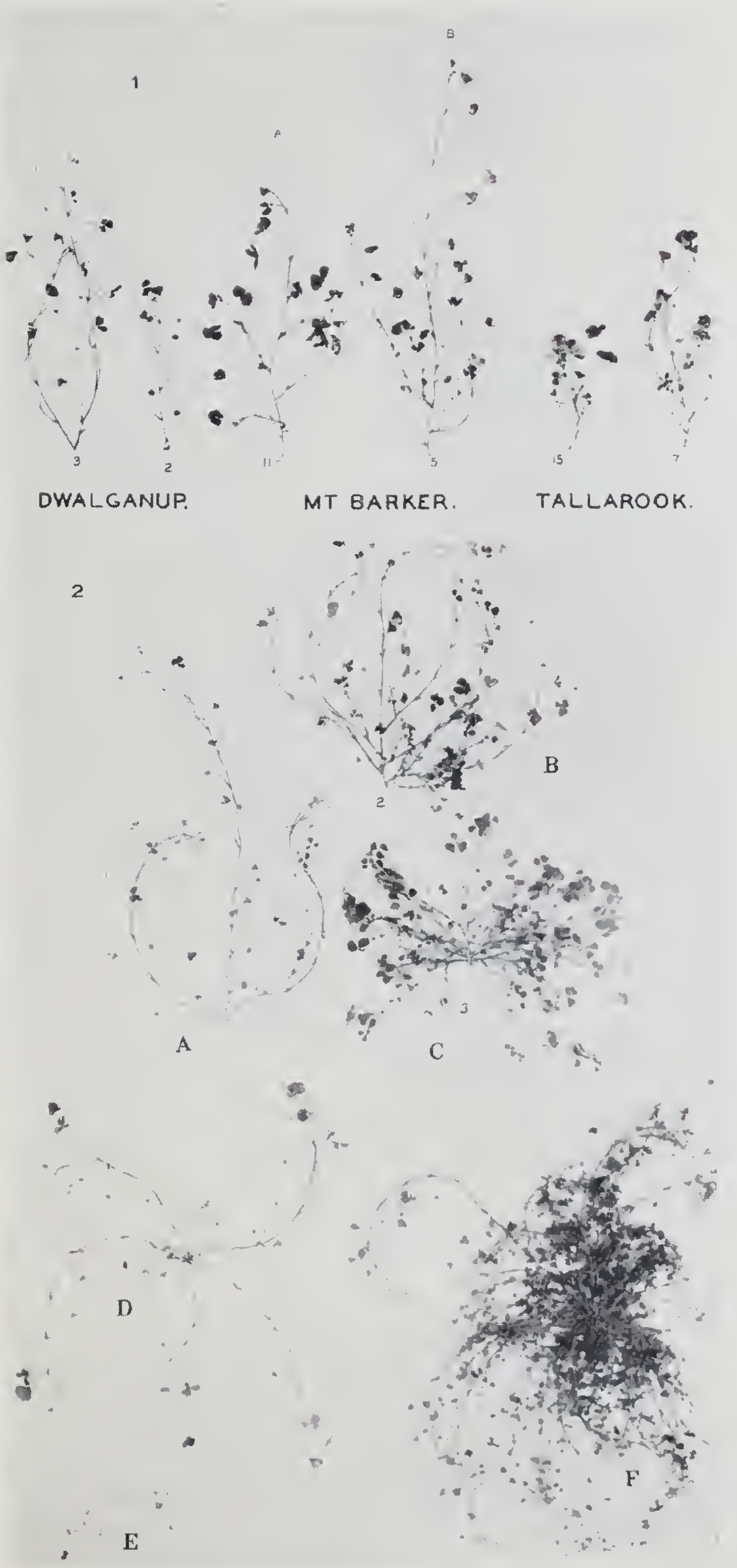
1. Plant very vigorous; runner 25–35 cm. long, lax, almost virgate; leaflets large, 2.5 cm. long and 3 cm. wide, distinctly toothed; peduncle long, but only as long or shorter than petiole; head with 2–3 large florets; burr the size of a pea; corolla pinkish-striped; calyx teeth ciliate. Mostly in Southern Mediterranean districts, but often more northerly. "Plant worth investigation." Variety *oxaloides* Rouy.

2. Like var. oxaloides, but with dark violet flowers; corolla 14–18 mm. long 3–4 times as long as calyx teeth, which are longer than corolla tube. Palestine, on light, stony places, occurring with var. oxaloides. Variety *Tel-Avivensis* Eig.

Occurrence of species—British Isles, France, Iberian Penin., Italy, including Islands, Balkan Penin., including Islands, Krun, Caucasus, Asia Minor, Persia, Syria, North Africa, Canary Islands, Madeira.

References.

1. Adams, A. B.—Subterranean Clover. *J. Ag. W. Aust.*, i., p. 514. 1924.
2. ————, A Pink Flowered Subterranean Clover. *J. Ag. W. Aust.*, vi., p. 370. 1929.
3. ————, A New Strain of Early Subterranean Clover. *J. Ag. W.A.*, vii., p. 540. 1930.
4. ————, An Early Strain of Subterranean Clover. *J. Ag. W. Aust.*, ix., p. 512. 1932.
5. ————, Subterranean Clover. *J. Ag. W. Aust.*, xi., p. 592. 1934.
6. ————, Response of Different Strains of Subterranean Clover to Seasonal Conditions. *J. Ag. W. Aust.*, xi., p. 596. 1934.
7. ————, Carne, W. M., and Gardner, C. A.—Subterranean Clover. *J. Ag. W. Aust.*, iv., p. 524. 1927.
8. Beggs, J. P.—Subterranean Clover Survey in Canterbury and North and Central Otago. *J. Ag. N.Z.*, lvii., p. 17. 1938.
9. Davies, W.—Observations on Subterranean Clover. *J. Min. Ag* xxxiv., p. 455. 1927.
10. Donald, C. M., and Smith Neal, C. A.—Strain in Subterranean Clover. *J. C.S.I.R.*, x., p. 277. 1937.
11. Drake, F. R.—Bacchus Marsh Subterranean Clover. *J. Ag. Vic.*, xxxviii., p. 108. 1940.
12. Forster, C., and Vasey, A. J.—Response of English and Australian Wheats to Length of Day and Temperature. *J. Ag. Vic.*, xxxiii., p. 352. 1935.
13. Gardner, C. A., and Dunne, W. M.—Subterranean Clover. *J. Ag. W. Aust.*, x., p. 42. 1933.
14. Hamner, K. C., and Bonner, J.—Photoperiodism in Relation to Hormones as Factors in Flower Initiation and Development. Bot. Gaz., C., p. 388. 1938.
15. Harrison, J. E.—"Tallarook" Subterranean Clover. *J. Ag. Vic.*, xxxiv., p. 541. 1935.
16. Levy, B. E., and Gorman, L. W.—Strain in Subterranean Clover. *J. Ag. N.Z.*, liv., p. 82. 1937.
17. Muratova, V.—Materials to Determination of the most important Forage Vetches. *Bull. App. Bot. and Pl. Br.*, xvi., p. 99. 1926.
18. Post, G. E.—Flora of Syria, Palestine and Sinai. 1., p. 34.
19. Radel, L. H.—Subterranean Clover. *J. Ag. Tas.*, vi., p. 16. 1935.
20. Roberts, R. H., and Struckmeyer, B. E.—Further Studies on the Effects of Temperature and other Environmental Factors on Photoperiodic Responses of Plants. *J. Ag. Res.*, lix., p. 699. 1939.
21. Trumble, H. C.—Climatic Control of Agriculture in S. Australia. *Trans. Roy. Soc. S. Aust.*, lxi., p. 41. 1937.
22. ————, Climatic Factors in Relation to the Agricultural Regions of Southern Australia. *Trans. Roy. Soc. S. Aust.*, lxiii., p. 36. 1939.
23. Ullman, W.—Beobachtungen über den Boden früchtigen Klee, seine Anbau und Zuchmöglichkeiten im Deutsche Reiche. *Pflanzenbau*, x., pp. 313, 359. 1934.
24. Vavilov, N.—Studies on the Origin of Cultivated Plants. *Bull. App. Bot. and Pl. Br., Leningrad*, xvi., p. 143. 1926.
25. Wexelsen Haakon.—Chromosome Numbers and Morphology in *Trifolium. Uni. Cal. Pubs. in Ag. Sc.*, ii., No. 13, p. 355.



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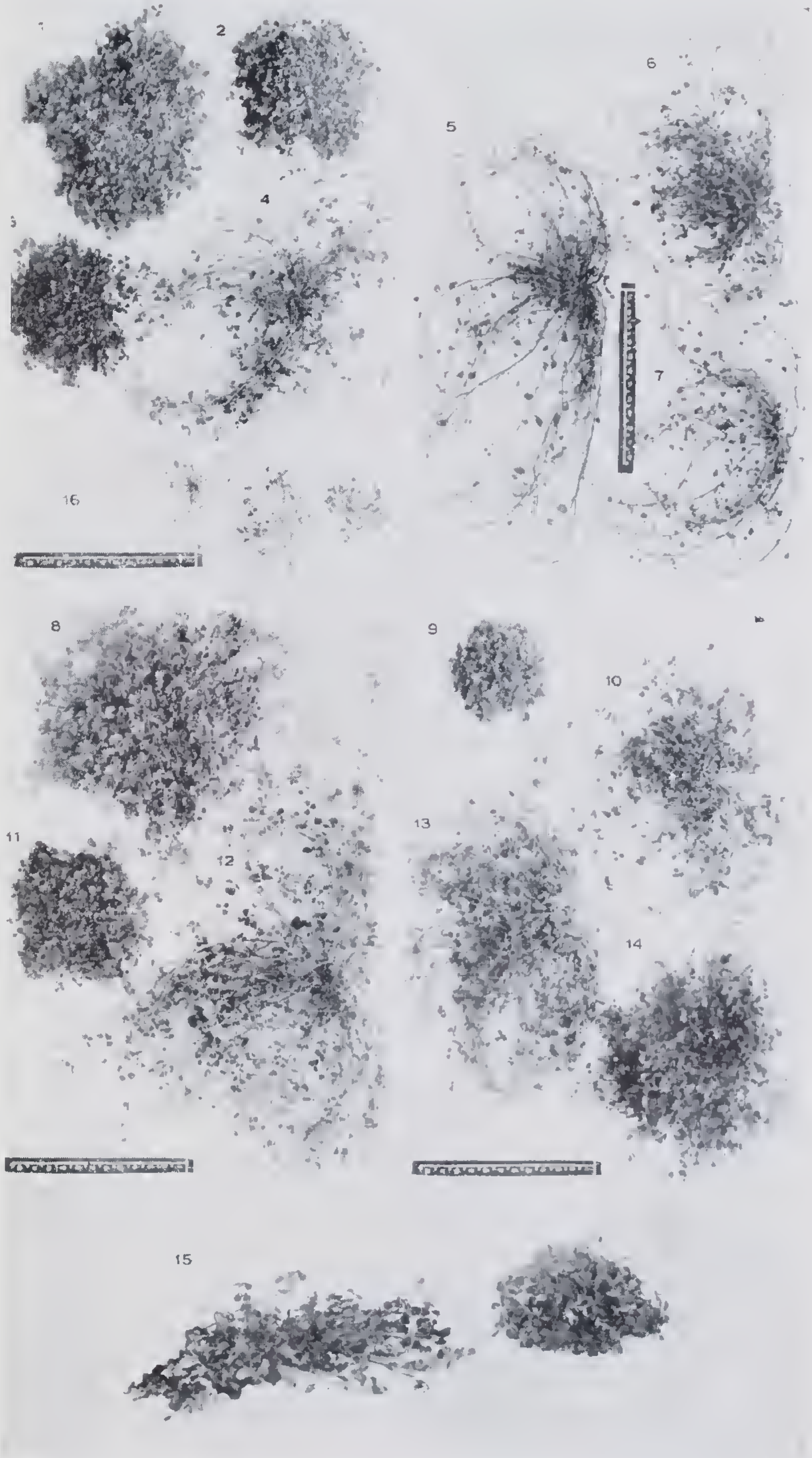

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